EVALUATION OF THE SUBGINGIVAL MICROBIOME IN PERIODONTAL HEALTH AND CHRONIC PERIODONTITIS USING NEXT GENERATION SEQUENCING TECHNOLOGY

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"EVALUATION OF THE SUBGINGIVAL MICROBIOME IN PERIODONTAL HEALTH AND CHRONIC PERIODONTITIS USING NEXT GENERATION SEQUENCING TECHNOLOGY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. K.V. ARUN, M.D.S.**, Professor and Head, Department of Periodontology, Ragas Dental College and Hospital, Chennai.

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CERTIFICATE

This is to certify that this dissertation titled **"EVALUATION OF THE SUBGINGIVAL MICROBIOME IN PERIODONTAL HEALTH AND CHRONIC PERIODONTITIS USING NEXT GENERATION SEQUENCING TECHNOLOGY"** is a bonafide record of work done by **Dr. Kalaivani .B** under my guidance during the study period 2014-2017.

This dissertation is submitted to **THE TAMILNADU DR.MGR MEDICAL UNIVERSITY** in partial fulfilment for the degree of **MASTER OF DENTAL SURGERY, BRANCH II- PERIODONTOLOGY.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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Introduction

INTRODUCTION

Microbial antigens are considered to play a crucial role in the initiation of the chronic inflammatory responses that lead to periodontal disease[104]. The etiological role played by plaque in the initiation and progression of periodontal disease of periodontal disease was clearly established following Loe's experimental gingivitis studies.[67]

Several models have been proposed to explain the exact role played by plaque in periodontal disease. The Non-specific plaque hypothesis postulated that it was the quantity of plaque that determined pathogenicity without discriminating between the levels of individual bacteria and their virulence. According to Theilade (1986)[113], periodontal disease occurred when plaque content with its toxins and metabolic products in totality exceeded the capacity of the host response.

The specific plaque hypothesis, proposed by Walter J.Loesche[68], stated that the quality and not the quantity of plaque mattered as only certain microorganisms in plaque were thought to be pathogenic. It was postulated that specific bacteria were associated with specific types of periodontal disease such as Aggregatibacter actinomycetocomitans (previously known as Actinobacillus actinomycetocomitans) with localized aggressive periodontitis (previously known as juvenile periodontitis).

Following the limitations of above mentioned hypothesis, Marsh proposed the 'Ecological Plaque Hypothesis', wherein the biofilm was considered to be of paramount importance. Environmental perturbations were considered to lead to the development of the subgingival biofilm and subsequently periodontitis.[73]

Technological advances in DNA sequencing and bioinformatics tools led to the development of the Omics technologies. It was subsequently realized that every individual has a specific microbiome and a metagenome that is unique to that person. The development of 16s rRNA and subsequently the Next Generation Sequencing technologies lead to further characterization of the microbiome.

Human Oral Microbiome Database (HOMD) currently lists the entire set of organisms present in the oral cavity as a whole. Several ecological niches have been identified within the oral cavity, each with a distinct microbiome of its own[20]. Several anatomical (such as the epithelium of mucosal surfaces) physiological (fluid compartments such as saliva, GCF) and environmental (PH, oxygen tension, nutritional characteristics) factors have been described as being involved in the development of these distinct microbiomes[23].

In the periodontal environment it has been reported that the supragingival and subgingival microbiome show distinct characteristics. The supragingival microbiome is dominated by bacterial populations that favour

aerobic, carbohydrate rich, neutral to alkaline PH environments. Conversely, subgingival microbiome is dominated by bacteria that thrive in anaerobic, protein rich, and acidic PH environments. Several studies have characterized the subgingival microbiome and well over seven hundred bacterial species have been identified, several of which are yet to be cultured[26,90,112]. It is also recognized that many bacterial species remain unidentified to date.

Currently, the pathogenesis of periodontal diseases is explained by "The Polymicrobial Synergy and Dysbiosis (PSD) Model"[40]. Hajishengallis proposed that dysbiosis and polymicrobial synergy were the key events that led to development of periodontitis[40]. According to this hypothesis, rather than individual bacteria, the biofilm as a whole was thought to be either health associated or disease associated. In a health associated microbial environment, there was considerable antagonism, as a result of which the virulence of the microbial population was suppressed and effectively countered by the host response. In disease states, dysbiosis occurred, where the microflora acted in a synergistic manner to enhance virulence of the biofilm and subvert the host response. He proposed that keystone pathogens such as Porphyromonas gingivalis, may play a role in this dysbiotic change, while pathobionts such as gram negative anaerobes and gram positive anaerobes, enhance non protective pro inflammatory responses that eventually damage host tissues.

Other than individual variations in the microbiome, considerable differences have been reported to exist across populations and ethnic groups.[87]

A variety of dietary and lifestyle patterns are thought to influence these racial and population based differences.

The subgingival microbiome in Indian population is yet to be characterized. The previous literature regarding microflora in Indian patients have all used closed ended RT- PCR techniques that are unable to identify the microbiome as a whole.

The current study was undertaken, as a first of its kind, to study the subgingival microbiome of Indian patients with periodontal disease using Next Generation Sequencing Technology.

Aim and Objectives

AIM AND OBJECTIVES

- To characterize the subgingingival microbiome in periodontal health and chronic periodontitis using NGS technology
- To compare the subgingival microbiome in periodontal pockets with that in healthy gingival sulcus.

Review of Literature

REVIEW OF LITERATURE

Human body bacterial cells are estimated to outnumber human cells by 10-times and that bacteria contribute more than 1,000,000 genes to the body while human (host) DNA contributes approximately 25,000 genes (Morgan 2012)[80]. The complex human microbiome represents approximately 90% of the cell count in and on the human body (Gill et al., 2006)[33]. These microorganisms contribute their genome, known as the metagenome, to the human body, multiplying human genes by approximately 100 times (Turnbaugh et al, 2007; Ling et al, 2010; Rajendhran and Gunasekaran, 2010)[118,65,112]. The activity of the microbiome and, specifically, the expression of its metagenome provide the human with resources and traits that did not originally evolve with the body (Rajendhran and Gunasekaran, 2010)[112]. For example, the microbiome contains genes that allow humans to digest certain plant polysaccharides (Rajendhran and Gunasekaran, 2010)[112].

There are various microhabitats throughout the body that contribute to the overall microbiome. The mouth, skin, gut, etc. each contains its exclusive microbiome and metagenome (Badger et al, 2011; Sonnenburg and Fischbach, 2011)[5,107]. Each microhabitat maintains a unique ecosystem with distinct atmospheric and nutritional compositions that provide a setting for symbiotic interactions among the various microbes within that ecosystem and the host. Microbiomes from the same location on the body are more similar among

different individuals than microbiomes from different locations on the same individual (Sonnenburg and Fischbach, 2011)[107].

The human microbiome can be classified into a core microbiome and a variable microbiome (Turnbaugh et al, 2007)[118]. The core microbiome is shared among all individuals and is comprised of the predominant species that exist under healthy conditions at different sites of the body (Turnbaugh et al, 2007; Zaura et al, 2009; Sonnenburg and Fischbach, 2011)[118,128,107]. The variable microbiome is exclusive to the individual and has evolved in response to unique lifestyle, and phenotypic and genotypic determinants. Although individuals share microbiota at similar sites of the body, there are varying differences at the species and strain level of the microbiome that can be as inimitable to the individual as is the fingerprint (Dethlefsen et al, 2007)[22].

The concept of human oral microbiome

The microorganisms found in the human oral cavity have been referred to as the oral microflora, oral microbiota, or more recently as the oral microbiome. The term microbiome was coined by Joshua Lederberg "to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease" (Lederberg)[62]. The human oral microbiome is all the microorganisms that are found on or in the human oral cavity and its contiguous extensions (stopping at the distal oesophagus). This term has been adopted by the Human Microbiome Project and considered as

the favoured nomenclature to define the complex oral bacterial community, their genetic elements and environmental interactions, which may be involved in disease (Dewhirst et al., 2010)[23].

Members of the human oral microbiome were among the first bacteria ever to be observed. In 1683, Antonie van Leeuwenhoek used his microscope to observe a large number of what he named "animalcules" in scrapings taken from his teeth. Over 200 years later, the seminal work of Koch, Pasteur and their contemporaries identified the animalicules as microorganisms and the first isolates of cultivatable members of the oral microbiome were studied in the laboratory.

It is now well recognised that the oral cavity supports one of the richest and most diverse of all the microbial communities that thrive on the human body, second only to the lower gastrointestinal tract (Peterson et al., 2009)[92]. This diversity is mainly because of the unusual tissue types that exist in the mouth; teeth are the only example within the body of a hard tissue being naturally exposed to the external environment. Also, teeth are not shed or turned over in the manner of soft tissues or epithelia. Therefore, the oral microbiota has evolved mechanisms to exploit environments that are not experienced by other microbiota (Avila et al., 2009)[4]. The architecture of teeth and their juxtaposition with other teeth and supporting soft tissues provide various niches which are exploited by the microbiome. An overgrowth of

microbiome constituents yields dental plaque, which is commonly associated with oral diseases such as caries and periodontal diseases.

The oral microbiota comprises of bacteria, fungi, archaea and viruses. Most research to date has focussed on the bacterial component of the microbiota (Kolenbrander, 2000; Marsh, 2005; Siqueira and Rocas, 2009)[56,73,100], although exploration of other components including viruses and fungi have been reported (Ghannoum et al., 2010; Wylie et al., 2014)[31,125].

Several lines of evidence indicate that bacteria are necessary for the development of inflammation in the periodontal tissues. Bacteria were implicated in periodontal disease with the observation that administration of penicillin inhibited periodontitis in laboratory animals (Mitchell and Johnson, 1956)[75], and the infectious nature of periodontitis was demonstrated by its transmissibility in animal models (Keyes and Jordan, 1964)[53]. The classic experimental gingivitis studies in humans by Loe et al. gives evidence of the resolution of inflammation after periodontal treatment involving mechanical debridement and animal models showing lower levels of bone loss in germ-free and antibiotic-treated animals (Loe et al 1965, Hajishengalis 2011).[67,39]

Role of plaque in periodontitis

Loe's experimental gingivitis recognized the etiologic role of plaque in periodontal disease and firmly established it was involved in the initiation and

progression of periodontal diseases. The ideas about how changes in dental plaque relate to a shift from oral health to disease have changed over time.

Non-specific Plaque hypothesis

The Non-specific plague hypothesis was based on work of researchers Black (1884)[10] and Miller (1890).[74] This hypothesis postulates that it was the quantity of plaque that determines the pathogenicity without discriminating between the levels of virulence of bacteria. According to Theilade 1986, when plaque content with its toxins and breakdown products exceeded the capacity of host response, disease occurs.[115] He also stated that all bacteria in plaque contribute to the virulence of the microflora by having a role in either colonisation, evasion of the defense mechanism, and/or provocation of inflammation and tissue destruction.

Non-specific plaque hypothesis is valid for the development of gingivitis but not for the development of periodontitis, which is a multifactorial disease (Page RC 1997)[87]. This concept also failed to explain why all gingivitis not progress to periodontitis and why some individuals with increased plague showed little overt periodontitis and some individuals with very little plaque manifested with aggressive and advanced forms of periodontitis (Socransky 1994)[10]. And also site specificity of the disease is inconsistent with the concept that all plaque are equally pathogenic.

Specific Plaque Hypothesis

The specific plaque hypothesis, proposed by Walter J. Loesche, stated that the quality and not the quantity of plague mattered as only certain microorganisms in plaque were thought to be pathogenic. When these specific bacteria increased in number, virulence factor released by them would lead to periodontal diseases. For example, Aggregatibactor actinomycetacomitans was identified as a specific pathogen in localized aggressive periodontitis (Newman MG 1976, Slots 1976)[83].

Following the development and maturation of dental plaque, with increase in probing depth, oral microbial flora specifically changes from gram-positive aerobic species to gram-negative anaerobic species (Socransky 1998, Marsh PD 2011).[104,73]

Socransky and Haffajee in 1998 identified specific microbial groups with dental plaque. Six inter-related groups were reported.[104] The yellow, green and purple complexes were the early colonizers that favour the colonization of orange and red complexes. Red complex bacteria without orange complex colonization were not usual. The red complex bacteria included Bacteroides forsythes (now Tannerella forsythia), Porphyromonas gingivalis and Treponema denticola and they were significantly associated with periodontitis. This failed to explain why the putative periodontal pathogens like Porphyromonas gingivalis, Tannerella forsythia are frequently found in healthy periodontal sites.

Ecological Plaque Hypothesis

Ecological plaque hypothesis was proposed by Philip D. Marsh in 1994.[73] This hypothesis stated that the disease is the result of an imbalance in the total microflora due to ecological stress, resulting in an enrichment of some oral pathogens or disease related organisms. More specifically, this hypothesis proposes that the nonspecific accumulation of plaque leads to inflammation within the gingival tissues and to the development of gingivitis. This leads to environmental changes within the gingival sulcus, which in turn favour the growth of gram-negative and proteolytic species of bacteria. These changes lead to further inflammatory and immune mediated tissue changes, further environmental changes and tissue destruction, culminating in a predominance of periodontal pathogens and a greater degree of tissue damage.

Keystone Pathogen Hypothesis

The Keystone Pathogen Hypothesis indicates that certain low-abundance microbial pathogens can cause inflammatory disease by increasing the quantity of the normal microbiota and by changing its composition (Hajishengallis et al., 2012)[40]. When disease develops and advanced stages are reached, the keystone pathogens are detected in higher numbers (Socransky et al., 1998)[104].

Polymicrobial Synergy and Dysbiosis Model

Recently described Polymicrobial Synergy and Dysbiosis (PSD) model of pathogenesis states that periodontitis is initiated by a broadly based dysbiotic, synergistic microbiota as against the traditional view that it is caused by a single or several periopathogens like red complex bacteria (Hajishengallis et al, 2012)[40]. This dysbiotic, synergistic microbiota alter host-microbe homeostasis and facilitate its transition to a chronic inflammatory state. Thus, the whole microbial community drives disease progression.

Tissue destruction, however, is mediated by the host and it is, therefore, the interplay between the subgingival community of microorganisms and local immune responses that ultimately drives bone and connective tissue attachment loss(Lamonte, Hajishengalis 2015)[61].

Subgingival Microbiome

The subgingival microbiome is the community of microorganisms inhabiting the subgingival environment. Subgingival microbiome has been the subject of investigation for many decades. Subgingival microbiota and its complexity has been recognized since the 1st microscopic examination of this ecosystem by Van Leeuwenhoek in 1683 (Tal, 1980)[109]. Since that time, numerous studies have evaluated the composition of plaque using light and electron microscopy, cultural techniques and immunologic or DNA probe techniques. All techniques reinforce Van Leeuwenhoek's initial observation

that subgingival plaques are comprised of a large complex mixture of bacterial species.

The microbial composition of subgingival plaque at periodontally diseased sites has been extensively studied (Haffajee & Socransky, 1994), Zambon, 1996).[36,127] A series of cultural studies of subgingival plaque taken from subjects with different forms of periodontal disease and health reported a shift in the subgingival microbiota as the periodontium progressed from health through gingivitis to periodontitis (Moore & Moore 1994).[77]

Although subgingival bacteria are the major cause of periodontal diseases, more than one-half of subgingival bacterial species or phylotypes are not readily cultivable, which presents an obstacle to fully understand the causal relationships between subgingival bacteria and periodontitis. To overcome the difficulties and limitations associated with cultivation, culture independent methods based on amplification and sequencing of bacterial genomes have been developed to identify thousands of different bacteria in a single sample.

Liu et al and Chen et al. investigated bacterial diversity between periodontal health and disease status using 16S rRNA amplicon sequencing and showed that there is a shift in the composition of the oral microbiota between healthy and diseased samples.[66,14]

APPLICATION OF MICROBIOLOGICAL TECHNIQUES TO PERIODONTAL DIAGNOSIS

The earliest record of the use of the microscope for examination of gingival crevicular contents in health and disease can be traced to experimental microscopy in the seventeenth century. In 1683 Antonie van Leeuwenhoek described 5 types of animalcules obtained from scrapings on human teeth in a letter to the Royal Society. He then related the increased numbers of these animalcules to poor hygienic oral conditions and dental disease. He observed that good hygiene reduced the numbers of these microorganisms. The pioneers who formed the foundation for the new discipline of medical microbiology with their investigations were Louis Pasteur and Robert Koch.

W.D. Miller, from the United States working in Robert Koch's laboratory in Berlin, described the microbial contents of the human mouth. He hypothesized that periodontal infection was nonspecific and depended on host response as well as microbial pathogenicity.[74] This nonspecific hypothesis persisted for more than 50 years. Cook, a contemporary of Miller, supported then on specific hypothesis and stated that his investigation also failed to fulfil the requirements proposed by Koch for the isolation of a disease-producing microorganism. Several bacteriological investigations were carried out but were unable to obtain pure cultures from deep pockets.

The first attempt at postulating a specific causative organism occurred in 1915, when Bass & Johns proposed that an amoeba (Entamoeba gingivalis) as the causative agent for pyorrhoea. Smears of plaque collected from periodontal pockets, stained for amoeba was then used as a diagnostic tool in periodontal treatment. Colyer (1910)[19], had proposed the use of dark field microscopy for the evaluation of pocket microorganisms. Theodore Rosebury (1930) conducted a series of experiments to isolate the bacteria of etiological importance in periodontal disease. He concluded that the etiology for periodontitis was nonspecific and dependent on local and systemic factors controlling host resistance. Keyes (1965)[53] proposed periodontal diagnosis using phase contrast microscopy to identify bacterial morphotypes. This was supported by other investigators using both phase contrast and dark field microscopy to identify morphologically the microorganisms associated with disease activity. Cultural studies have also focused on the identification of specific microorganisms associated with clinical disease activity. Several bacterial species have been reported to be associated with specific clinical diagnoses, such as rapidly progressive periodontitis and juvenile periodontitis..

Recently, several studies employing DNA-based technologies revealed great richness of the healthy core oral microbiome, either via cloning and sequencing approaches of microbial 16S rDNA (Aas et al., 2008, Preza et al., 2008, Riggio et al., 2008)[1,93,95] or by revolutionary next generation sequencing methods (Keijser et al., 2008; Zaura et al., 2009).[51,128]

The Culture Based Methods

Traditionally identification of the species in any given sample was achieved by growing it in vitro on suitable media. Culturing can be done on selective and non-selective media. Blood agar is a common non-selective medium as it allows growth of a broad spectrum of organisms (Samaranayake, 2002)[98]. More specific media include Gram negative anaerobic medium supplemented with vancomycin to selectively allow growth of Gram negative anaerobic rods while inhibiting Gram positive bacteria (Samaranayake, 2002).[98] Another example of a selective medium is Staphylococcus spp. isolation on mannitol salt medium, as fermentation of this salt by Staphylococcus aureus will turn the medium from pink to yellow (Samaranayake, 2002).[98] Laboratory culturing under special conditions and using a range of media has allowed isolation of a diverse range of bacteria. However, it is well recognized that the main drawback of this method is its narrow spectrum. It has been estimated that 50% to 60% of distinct bacterial phyla in oral cavity still have no cultivable representatives (Kolenbrander, 2000, Vartoukian et al., 2010, Siqueria et al., 2013).[56,120,100] Moreover, the culture-dependent technique is expensive, sensitive and needs a highly skilled individual.

There is a growing need for developing improved methods to cultivate and characterize the as-yet-uncultivated portion of the oral microbiome so as to unravel its role in health and disease (Siqueria et al., 2013)[100]. Theoretically,

all bacteria can grow under proper nutritional and physicochemical conditions (Clarridge et al., 2004)[18]. However, development of new improved culture media is still a challenging goal and is mainly due to the highly diverse microbial community present with each member having different nutritional requirements (Tian et al., 2010)[117]. Siqueria et al. (2013)[100] has recently suggested a list of recommendations in order to cultivate the yet-uncultivated bacteria, such as the use of culture media with little or no added nutrients and addition of specific growth factors in the culture media. A very interesting strategy to ensure the availability of natural growth factors is to perform incubation in the natural environment using special devices (Kaeberlein et al., 2002, Gavrish et al., 2008, Sizova et al., 2012)[49,30,101] such as a diffusion chamber (Kaeberlein et al., 2002, Bollmann et al., 2007)[49,11] or a hollow fibre membrane chamber (Aoi et al., 2009)[3], which allow diffusion of important growth factors from a natural environment to the culture via a special membrane (Siqueria et al., 2013)[100].

Though considered gold standard for identification and analysis of bacteria, bacterial cell culture has its own limitations. Only viable bacteria will grow, and stringent transport and storage conditions will be needed to identify those bacteria that are 'cultivable'. Those that are not cultivable would remain unidentified. This could be due to the fact that many species in the oral cavity are fastidious and require the presence of other organisms and very specific growth conditions. If those conditions, that could even be unknown and

unexplored, are not provided, then the bacterial species that are the most fastidious will simply not grow in culture and will remain unidentified. Bacterial identification using newer molecular methods will surpass the limitations associated with culture based methods for identifying bacteria. However, cell culture is still essential to assess bacterial sensitivity to antibiotics and, also for verifying the presence of known species

Immunologic and enzymatic assays

The antibody-based detection systems help in more accurate detection of targeted species. Raising of antibodies to each species of interest needs growing the organism in culture before inoculating an animal and raising antibodies to the bacterial antigens. This method was used to explore the prevalence of target species in the healthy and disease population and to identify the changes in these species either naturally or in response to treatment (Wolff, L.F., et al, 1993, Loesche, W.J., et al.,1992)[123,69].

Limitation of immunological assays is that the target organism has to be cultured to raise antibodies against it. This makes this method useful only for cultivated species. The antibodies cross reactivity can be tested only on cultivated species and cannot be done on uncultivated or unknown species.

DNA – DNA hybridization or checkerboard

DNA-DNA hybridization is a molecular approach that has been used in a large number of studies. This method detects bacteria based on hybridization

of target species to labeled genomic DNA that has been attached to nylon membranes previously. The levels of a limited number of species have been studied with this method in adult periodontitis, periodontal health, refractory periodontitis and response to therapy (Loesche, W.J., et al.,1992, Gmur, R.1990, Ximenez-Fyvie, L.A., 2000, Socransky 2002, Haffajee,1998, Feres, M., et al.,2001)[69,34,126,106,37,27]. They used data from population based studies and grouped 40 species that were found in clusters or 'complexes' by comparing the levels of these species in health and disease. Three species, Porphyromonas gingivalis, Tannerella forsythia (Bacteroides forsythus) and Treponema denticola, were found to be significantly increased in disease when compared to health. These three species were grouped into the 'red complex' bacteria. Chairside Diagnostic tools were available based on assays that detect these species. Red complex bacteria were thought to be the primary etiological agents for more than 30 years and therapeutic intervention to eradicate these species were also considered. DNA-DNA hybridization has the advantage of detecting multiple species from each sample simultaneously. But this method is also depends on culture technique to cultivate the target species for creating genomic probes. Like antibody-based assays, cross reactivity can be verified only with cultivated species and so specificity of the probe is an unknown variable. Selected species may not be representative of the entire microbiome and while result interpretation this fact should be considered.

Polymerase chain reaction

Kary Mullis in1993 (Mullis et al., 1987)[82] first developed the polymerase chain reaction (PCR) and this technique amplifies specific genes or parts of genes which then to be used to identify the bacterial species they originated from. PCR-based methods were used by researchers in their studies to detect specific species directly from oral samples. They focused mainly on the identification of a few species associated with the putative periodontal pathogens, such as Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, and Aggregatibacter actinomycetemcomitans (de Lillo A,2004, Leys EJ, 2002, Okada M, 2005, Sanz M, 2004, Tanner AC, 2006)[21,64,85,99]. In previous sequence analysis of 16S rRNA genes from oral microflora, a number of bacterial species were identified as candidates as putative pathogens for periodontitis, that includes the traditional species, such as P. gingivalis, T. denticola and T. forsythia (Paster BJ, 1998)[89]. Species specific PCR primers were designed and used in individual PCR reactions to detect the prevalence of target species in plaque samples of healthy subjects and diseased subjects (Kumar PS, 2005)[59]. These studies confirmed that several more species, including uncultivated, were associated with oral health or periodontitis.

Real -time polymerase chain reaction

Real-time PCR is also referred to as qPCR, qRT-PCR, RT-qPCR and kinetic PCR. The procedure relies on the same basic principles of PCR; the

additional feature is that the amplified DNA is detected and quantified simultaneously as the reaction progresses in real-time. Real-time PCR has been used to detect and quantify several periodontal pathogens including A. actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, the tetQ gene and total bacteria, in clinical samples.

Open-ended approaches

Open ended approaches allow identification even uncultivated and previously unknown species. These approaches are based on 16 S rRNA sequencing. This approach has been used to study the microbial population in different ecosystems, enabling the characterization of hitherto uncultivated microbial communities (Pace, N.R., 1997, Frank, D.N., 2003)[29,86]. Using this approach, the diversity of different colonization niches in the oral cavity has been explored (Paster, B.J., et al., 2002, Becker, M.R., et al., 2002)[90,7] .

16S rRNA SEQUENCING ANALYSIS

One of the introduced culture-independent approaches is based on amplification and analysis of the 16S rRNA genes in a microbiome sample (Spratt, 2004)[108]. 16S rRNA has proven to be the most useful phylogenetic marker to identify bacteria and to determine their evolutionary relationships. Ribosomal RNA gene is essential for life and present in all prokaryotes. It contains nucleic acid sequences with highly conserved and variable regions. The conserved regions are used to design universal PCR primers capable of

recognizing segments of the 16S rRNA gene sequence of all bacterial species. The hypervariable regions can be used as signatures to discriminate one species from another. 16S rRNA gene is large enough (about 1500 bases) to provide sufficient sequence variability among bacteria, thereby making comparisons possible at different taxonomic levels. This method is truly culture-independent in that bacteria can be identified within a sample without the need for culture.

NEXT GENERATION SEQUENCING TECHNIQUE

The next-generation sequencing is done by repeated cycles of polymerase-mediated nucleotide extensions or by machinery automated cyclical ligation of oligonucleotides (Mardis 2008, Voelkerding et al., 2009)[71,121]. Huge amount of nucleotide sequence output is given as millions of reactions occur in a massively parallel process in a single machine run. The three commonly used platforms for massively parallel DNA sequencing at present are the Roche/454 FLX (Life Sciences, Branford, CT, Margulies *et al*., 2005)[72] and Illumina/ Solexa Genome Analyzer (Illumina, San Diego, CA, Bentley DR, 2006, Korbel *et al*., 2007)[57] Applied Biosystems / SOLiD (Life Technologies, Carlsbad, CA, Mardis 2008, Voelkerding et al., 2009)[71,121]. The most recent powerful NGS platforms with a significant reduction of the run time and remarkable data output, include HiSeq and the Ion Torrent Personal Genome Machine (PGM) (Rothberg *et al.*, 2011)[96].

First-generation systems have two consistent themes, the ligation of DNA fragments with oligonucleotide adaptors and the fragments immobilization to a solid surface, such as a bead. The purpose of the adaptors are, to anchor the fragments to a solid surface and to serve as primers for amplification and/or sequencing.

In **Roche / 454**, in addition to the common theme, it is based on pyrosequencing technology. The protocol includes (i) clonal amplification of templates on beads; (ii) deposition of the beads onto picotiterplate wells; (iii) controlled delivery of deoxyribonucleotide triphosphates by laminar fluidics, and (iv)a high resolution charge-coupled device camera that detects the luminescent burst upon deoxyribonucleotide triphosphate incorporation. Advantage of 454 sequencing is its long read lengths (400–500 nucleotides) and the amount of sequence generated (0.5 Gb) (Mclean 2009)[70]. The long reads can handle repetitive regions better than other nextgeneration sequencing systems. A major weakness of the 454 sequencing system is that sometimes more than one nucleotide is incorporated in the DNA template during a cycle, making it difficult to resolve homopolymeric stretches of sequence (e.g. CCCCC or AAAAA).

SOLiD system can generate 4 Gb of sequence but the reads are only 35 nucleotides (Voelkerding KV, 2009)[121]. The two-base encoding system provides better sequence fidelity than the one-base next-generation sequencing systems. The weakness of the SOLiD system is, that it yields biased sequence

coverage in AT-rich repetitive sequences (Harismendy O, 2009)[41] and only 35% of the raw reads are useable, compared with 95% for the 454 system. Another weakness is it requires long run times.

Illumina/Solexa Genome Analyzer

Acquired by Illumina in 2006, this highly targeted NGS approach provides more sequence reads per run, than previous methods thereby allowing for more in depth coverage (Bentley, 2006; Korbel *et al*., 2007; Bentley *et al*., 2008)[8,57,9]. The Genome Analyzer uses a specific number of cycles, where fluorescently labeled reversible-terminator nucleotides are detected on clonally amplified DNA templates that are immobilized to an acrylamide coating on the surface of a glass flow cell (Bentley, 2006, Korbel *et al*., 2007; Bentley *et al*., 2008)[8,57,9].

In the Solexa system, the targets are amplified on a solid surface. After amplification, only one of the strands is sequenced with all four deoxyribonucleotide triphosphates present during sequencing (not one at a time, as in the case of the 454 system). Each deoxyribonucleotide triphosphate has a unique fluorophore. Reversible terminator nucleotides (also called cyclic reversible termination (Metzker 2010)[76] are used to prevent the insertion of multiple nucleotide bases in the same cycle. In detail, the DNA is fragmented and adaptor sequences are added to each end of the fragments. The fragments are then sent to a lawn of immobilized oligonucleotides that are grafted to the surface of a microfluidic chamber. The DNA templates are hybridized to the

immobilized oligonucleotides by the adaptors. Once attached, the DNA templates are copied using bridge amplification (Adessi C, 2010)[2]. Bridge amplification involves the tethered DNA template arching over and hybridizing to an adjacent anchored oligonucleotide, forming a bridge. Amplification of a single DNA molecule results in a cluster of molecules composed of the same sequence. Following amplification, the reverse strands of the DNA are denatured and washed away, resulting in clusters of unique immobilized ssDNA. DNA sequencing begins with the addition of polymerase, fluorescently labeled deoxyribonucleotide triphosphates and a primer that hybridizes to one of the adaptors. The incorporation of a complementary base results in a burst of light that is recorded by a charge-coupled device camera. Unlike the 454 sequencing system, the fluorophore is removed from the incorporated base, washed away and the cycle is repeated. This prevents the addition of more than one base per cycle.

The strength of the Solexa system is that it can generate 1.5 Gb of sequence per run with read lengths that range from 35 to 100 bases. Each run requires 3–5 days to complete (Rothberg JM, 2008)[96]. To deal with short read length, the confidence of the sequence reads is improved by using pair-end sequencing, which means that both ends are sequenced. However, the short read lengths tend to produce biased sequence coverage that occurs in AT-rich repetitive sequences- a weakness with the Solexa system (Harismendy O, 2009)[41].

Technical difficulties sometimes arise as the system can also be affected by dephasing noise that occurs when a complementary nucleotide is not incorporated or when the fluorophore is not properly cleaved at the end of the cycle – blocking the incorporation of the next nucleotide base. As a consequence, the sequence is out-of-phase for the remainder of the template (Dohm JC, 2008)[24].

THE HUMAN ORAL MICROBIOME DATABASE

HOMD is a web accessible resource for the investigation of oral microbe taxonomic and genomic information. Though human oral microbiome is the most studied human microflora, 53% of the species have not named yet and 35% of species are uncultivated. The uncultivated taxa are identified mainly by 16S rRNA sequence information. Human Oral Microbiome Database (HOMD) provides database for the more than 700 prokaryote species present in the human oral cavity based on a curated 16S rRNA gene-based provisional naming scheme. Currently, two primary types of information are provided in HOMD—taxonomic and genomic. Each of 16S rRNA phylotypes is given unique Human Oral Taxon (HOT) number. The HOT interlinks phenotypic, phylogenetic, genomic, clinical and bibliographic information for each taxon. A BLAST search tool is provided to match user 16S rRNA gene sequences to a curated, full length, 16S rRNA gene reference data set. For genomic analysis, HOMD provides comprehensive set of analysis

tools and maintains frequently updated annotations for all the human oral microbial genomes that have been sequenced and publicly released.

The basic list of oral bacteria came from the literature of Dzink, J.L., 1985, 1988, Sockransky, 1994, Tanner 1979,1998, Moore W.E., 1982, 1983, 1994.[25,26,105,110,111,76,77,78]

Sockransky determined the presence and levels of 40 subgingival taxa 13,261 plaque samples using whole genomic DNA probes and checkerboard DNA-DNA hybridization. 5 major complexes were consistently observed using any of the analytical methods.[105]

Tanner et al. compared the subgingival microbiota in periodontal health, gingivitis and initial periodontitis using predominant culture and a DNA probe, checkerboard hybridization method. The data suggest that Tannerella forsythia, Campylobacter rectus and Selenomonas noxia were major species characterizing sites converting from periodontal health to disease.[110,111]

Dzink et al. observed that proportions of Gram negative rods were higher in active periodontal disease sites than in inactive sites. Species which were found to be significantly elevated only in active sites were Bacteroides intermedius, fusiform Bacteroides, Actinobacillus actinomycetemcomitans and Wolinella recta. Fusobacterium nucleatum, Capnocytophaga gingivalis

and Eikenella corrodens were found in significantly increased proportions in active sites of some subjects and inactive sites of others.[25,26]

Moore WL et al., detected 509 different kinds of bacteria among 51,000 bacterial isolates from gingival crevices of 300 people. Of these taxa, 368 were detected more than once.[76,77,78]

Dewhirst, 2010, identified 1,179 taxa, of which 24% were named, 8% were cultivated but unnamed, and 68% were uncultivated phylotypes. Upon validation, 434 novel, non-singleton taxa were added to the HOMD.[23]

SUBGINGIVAL MICROBIOME – RELATED STUDIES

The subgingival microbiome is the microflora community that inhabits the subgingival environment is subject of investigation for several years. Before the advent of 16S ribosomal RNA (rRNA) gene sequencing, the techniques used in several studies were close ended. These methods could evaluate limited number of species and they were restricted to cultivable species. After the advent of 16S rRNA gene sequencing, the pioneer studies that investigated subgingival microbiome in health and under different periodontal conditions used Sanger sequencing, which was labour-intensive cloning process. The studies based on this were still not high throughput and lacked the sequencing depth to cover most subgingival diversity within samples.

Kroes et al. (1999)[58] evaluated the subgingival microbiome using 16S rRNA gene sequencing and Sanger sequencing. This was the first study that utilized 16S rRNA gene sequencing in subgingival microbiome characterization. 77 phylotypes were identified and 48 percent of which were novel.

Paster et al. (2001)[90] evaluated the subgingival microbiome using Sanger sequencing in different periodontal conditions. He identified a total of 347 phylotypes and 215 of which were novel.

Kumar et al. (2005)[59] evaluated the subgingival microbiome with Sanger sequencing in health and periodontitis. A total of 274 phylotypes were identified and this was the first controlled study comparing health and chronic periodontitis using 16S rRNA gene sequencing. Phylotypes associated with periodontitis were identified as Peptostreptococcus spp., Filifactor alocis, Megasphaera sp., Desulfobulbus sp., Dialister spp. Campylobacter spp., Selenomonas sp., Deferribacteres sp., Catonella sp., Tannerella forsythia, Streptococcus spp., Atopobium sp., Eubacterium sp. and Treponema sp. Phylotypes associated with health were Veillonella sp., Campylobacter gracilis, Campylobacter showae, Abiotrophia adiacens, Eubacterium saburreum, Gemella sp., Streptococcus sanguis, Streptococcus mutans, Capnocytophaga gingivalis, Rothia dentocariosa, Eubacterium sp. and Selenomonas sp.

High throughput sequencing allows direct sequencing of 16S rRNA gene circumventing cloning step. It also allows simultaneous characterization of subgingival microbiome composition of many samples, at a relatively low cost in a short time period and obtaining thousands of sequences per sample guarantee detection of most species present. The studies that used such technology are listed below.

Griff en et al., (2012)[35] compared health and periodontitis using high throughput sequencing. He found 16 phyla, 106 genera and 596 species .He showed that the health associated species are also present in disease, but suppressed.

Abusleme et al., (2013), with qPCR showed that 46 species-level phylotypes were enriched in periodontitis and 14 were enriched in health. He concluded that shifts from health to periodontitis resemble ecological succession without replacement of health-associated species. He defined core subgingival species as those present in a majority of subjects and at equal relative abundance in health and disease. He defined core subgingival species as those present in a majority of subjects and at equal relative abundance in health and disease. F. nucleatum was the most abundant core species.

Kistler et al., (2013)[55] identified species-level phylotypes positively and negatively correlated with gingivitis. Increased community diversity and significant shifts in microbiome structure after two weeks of oral hygiene abstention was reported.

Huang et al., (2014)[46] concluded that 15 genera could distinguish healthy and gingivitis samples with 74 percent accuracy. Temporal shifts in community structure were observed along the progression from naturally occurring gingivitis to healthy gingiva to experimental gingivitis.

Park et al., (2015)[87] showed distinct communities in health, gingivitis and periodontitis.

Hong et al., (2015)[45] concluded that no demographic or medical characteristics of periodontitis subjects were associated with specific microbial profiles. Two types of microbiome profiles were identified in periodontitis (clusters A and B) by clustering analyses of microbial abundance profiles. Type B communities showed increased proportions of certain periodontitis-associated organisms, such as Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola, and taxa recently linked to periodontitis. In subjects with type A communities had increased proportions of different periodontitis-associated species, health-associated species and core taxa (prevalent both in health and periodontitis). The cluster B community showed a positive correlation with periodontitis extent.

Kirst et al., (2015)[54] confirmed that microbial diversity was not significantly different between health and periodontitis but communities in health and periodontitis diff ered. 18 species-level phylotypes enriched in periodontitis and five enriched in health.

Chi-Ying Tsai (2015)[17], conducted a study with the aim of determining the subgingival microbiota in Taiwanese individuals with severe chronic periodontisusing a 16S rRNA metagenomic approach. He also demonstrated a microbial shift from health to disease.

Materials and Methods

MATERIALS AND METHODS

Study population

Considering the cost involved and sheer complexity of the technology used and data obtained microbiome studies are difficult to perform in large population. Our study utilized a sample of 8 patients as per previous studies by Zheng et al, Dzink et al., who used a similar sample size in their study.[129,26]

A total of 8 individuals seeking dental treatment in Ragas Dental College and Hospitals, Chennai, were involved in the present study, of which 4 were periodontally healthy individuals(control group) and 4 were chronic periodontitis patients(test group). A diagnosis of chronic periodontitis was determined based on the American Academy of Periodontology parameters.[113]

CONTROL Group consisted of 4 subjects with clinically non-inflamed, healthy gingiva (probing pocket depth {PPD} ≤ 3mm, no clinical attachment loss {CAL}, no bleeding on probing {BOP}).

TEST Group consisted of 4 subjects with chronic periodontitis with PPD ≥ 5mm and CAL ≥ 3mm in at least six sites.

The study protocol was explained, and written informed consent was received from each individual before clinical periodontal examinations and subgingival plaque sampling. Medical and dental histories were obtained.

INCLUSION CRITERIA

- Subjects exhibiting good general health
- Subjects meeting the criteria of periodontal health and disease as described above were included in this study.

EXCLUSION CRITERIA

- Patient with systemic disorders, such as diabetes mellitus or immunological disorders, HIV
- Patients on drugs that have potential to interfere with microbial characteristics such as immunosuppressant drugs or steroids.
- Patients with history of tobacco usage.
- Patients with history of periodontal treatment in the past 6 months.
- Patients under antimicrobial therapy for the past 6 months.

Subgingival plaque sampling

All examinations were performed by a single, calibrated examiner. For the diseased samples, the deepest pockets were selected and the sample was collected in a single Eppendorf tube. Supragingival plaque was first removed from the sample teeth with sterilized Gracey curettes. The site was then cleaned and isolated using cotton rolls and air dried gently. Another sterilized Gracey curette was inserted into the deepest part of the pocket and plaque was removed by applying a slight force toward the root surface. The tip of the curette was then inserted in the Eppendorf tube containing ionized molecular

water and shaken until the plaque was removed from the curette. For the healthy subgingival plaque samples, sites that did not exhibit any sign of inflammation and bleeding on probing were chosen. The same procedures were followed for the subgingival sampling from these sites.

The samples obtained were frozen and stored at -20°C until the sample collection period was completed. All the samples were collected within 2 days and then sent for processing so as to avoid any degradation.

DNA extraction, 16S rRNA amplification, library construction and sequencing

Genomic DNA was extracted from 8 subgingival plaque samples of periodontitis and health patients with the Fast DNA kit and the FastPrep24-5G instrument according to manufacturer's recommendations (MP Biomedicals, Santa Ana, CA).

Extracted DNA was purified with silica-based spin filters (FastDNA kit) and DNA was amplified using the 16S V3 (341F) forward and V4 (805R) reverse primer pairs with added Illumina adapter overhang nucleotide sequences.

Amplicon synthesis was performed using thermocycling with 8.5 µl of genomic DNA, 2 µl of amplicon PCR forward primer (2.5 µM), 2 µl of amplicon PCR reverse primer (2.5 µM), and12.5 µl of 2x KAPA HiFi HotStart Ready Mix (Kapa Biosystems) at 95 °C initial denaturation for 3 min,

followed by 25 cycles of 95 °C for 30 s, 62.3 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

Reactions were cleaned up with Agencourt AMPure XP beads (Beckman Coulter Genomics) according to the manufacturer's protocol. Attachment of dual indices and Illumina sequencing adapters was performed using 5 µl of amplicon PCR product DNA, 5 µl of Illumina Nextera XT Index Primer 1 (N7xx), 5 µl of Nextera XT Index Primer 2 (S5xx), 25 µl of 2x KAPA HiFi HotStart Ready Mix, and 10 µl of PCR-grade water (UltraClean DNA-free PCR water; MO BIO Laboratories, Inc., Carlsbad, CA, USA), with thermocycling at 95 °C for 3 min, followed by 8 cycles of 95 °C for 30s, 55 °C for 30s, and 72 °C for 30s, and a final extension at 72 °C for 5 min.

Constructed 16S metagenomic libraries were purified with Agencourt AMPure XP beads and quantified with Quant-iT PicoGreen and the KAPA Library Quantification Kit (KAPABIOSYSTEMS). Library quality control was performed with the Agilent Technologies 2100 Bioanalyzer to ascertain quality and average size distribution.

Samples were denatured and diluted to a final concentration of 10 pM with a 20 % PhiX (Illumina) control. Sequencing was performed using the Illumina Nextseq 500 System. All 8 samples were multiplexed and sequenced in a single lane on the NextSeq using 2 × 150 bp paired-end sequencing. Data analysis was done by using 16s metagenomics tool from Base Space Onsite.

Operational taxonomic units (OTUs) were assigned to each sequence using HOMD database.

Statistical analysis

Conventional statistical analysis cannot be done in this study due to individual variation in the subgingival community and all data was analysed as per previous studies (Kumar et al. 2005, Griffen et al.,2012, Liu et al.,2012).[59,35,66]

Circular maximum likelihood phylogenetic tree at the level of genus was constructed using iTOL and PhyloT tools as per Griffen et al.[35]

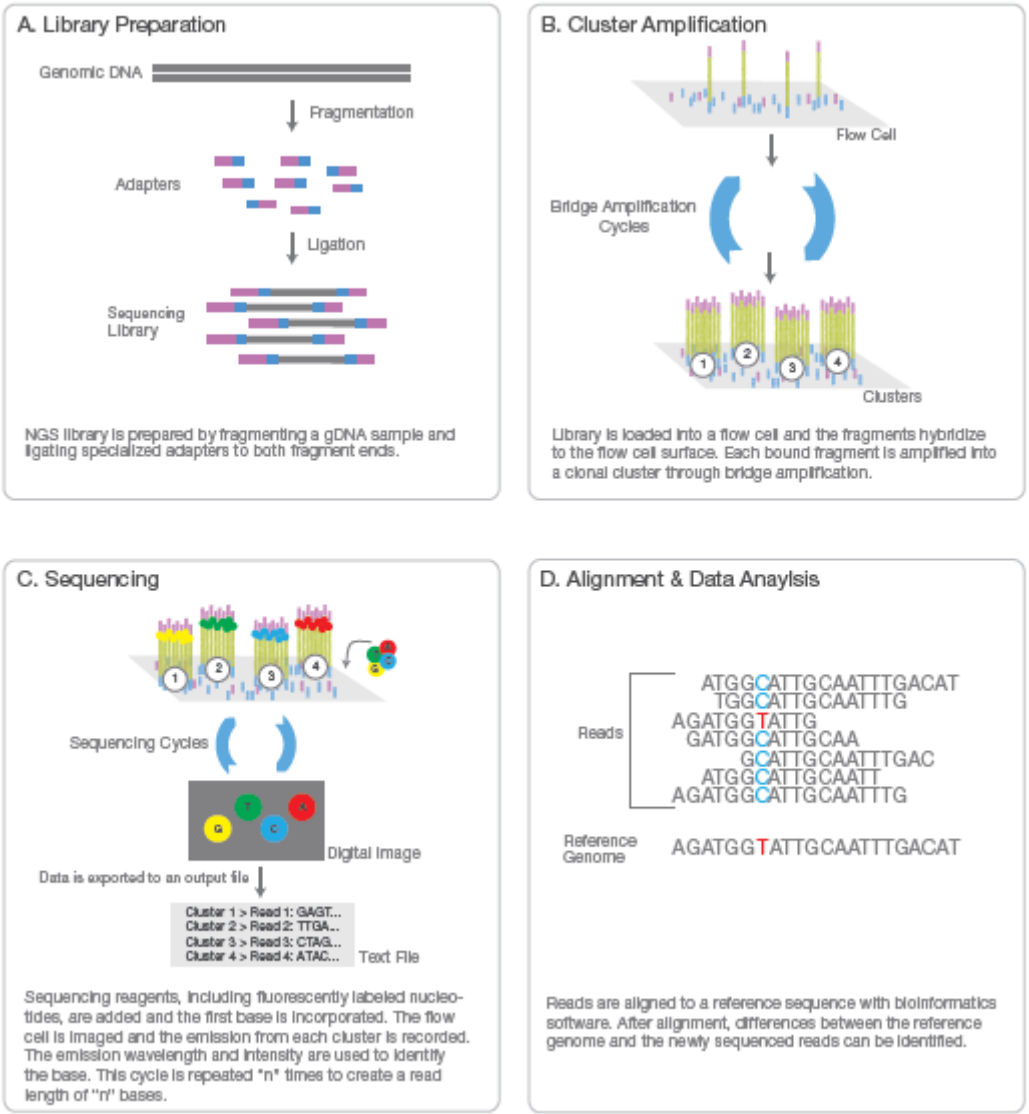
Photographs

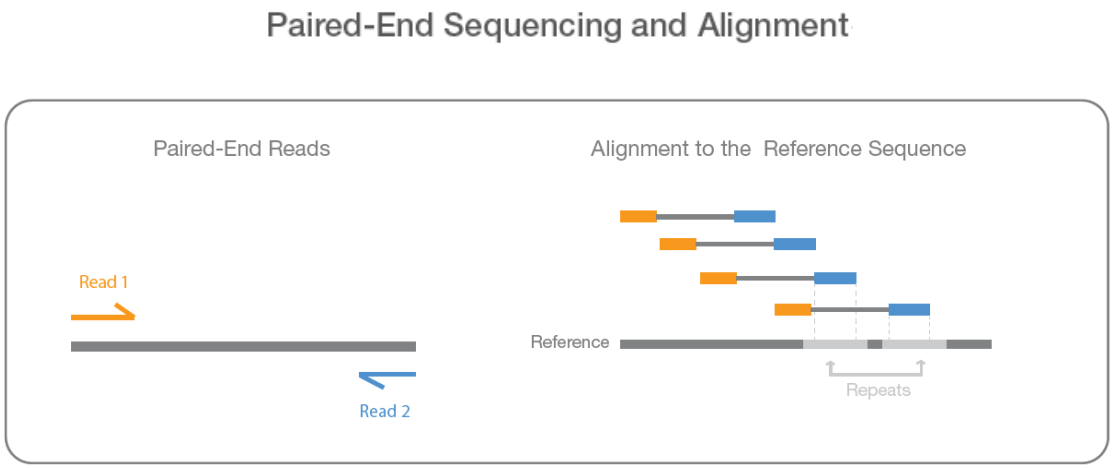
SAMPLE COLLECTION

ILLUMINA SEQUENCING

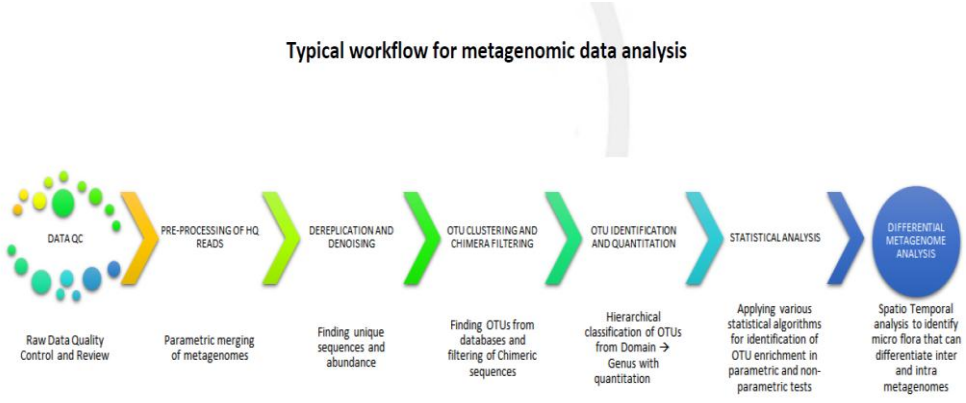
NextSeq Series

4 basic steps in Illumina NGS work flow




Paired-End Sequencing and Alignment
Paired-End Reads
Alignment to the Reference Sequence
Read 1
Read 2
Reference
Repeats


NGS Library Preparation Kits
Illumina Sequencing Instruments
Automated Data Analysis Tools
5–6 Hours
1–2 Days
1–2 Days


Typical workflow for metagenomic data analysis
DATA QC
PRE-PROCESSING OF HQ READS
DEREPLICATION AND DENOISING
OTU CLUSTERING AND CHIMERA FILTERING
OTU IDENTIFICATION AND QUANTITATION
STATISTICAL ANALYSIS
DIFFERENTIAL METAGENOME ANALYSIS
Raw Data Quality Control and Review
Parametric merging of metagenomes
Finding unique sequences and abundance
Finding OTUs from databases and filtering of Chimeric sequences
Hierarchical classification of OTUs from Domain → Genus with quantitation
Applying various statistical algorithms for identification of OTU enrichment in parametric and non-parametric tests
Spatio Temporal analysis to identify micro flora that can differentiate inter and intra metagenomes

Results

RESULTS

Eight subgingival samples consisting of four samples from periodontally healthy individuals (designated as (1S (H), 2S (H), 3(H), 4(H)) and four from chronic periodontitis individuals (designated as 5S (D), 6S(D), 7S(D), 8S(D)) were collected.

V3-V4 amplicons of 16srRNA gene were sequenced. The results obtained are represented according to classification system of bacteria.

I. Characterization of the subgingival microbiome

A total number of 27 phyla, 626 genera and 1278 species were identified as a whole.

Table 1: 27 phyla identified in the subgingival samples

1	Bacteroidetes	15	Caldithrix
2	Proteobacteria	16	Deferribacteres
3	Firmicutes	17	Thermodesulfobacteria
4	Fusobacteria	18	Acidobacteria
5	Thermi	19	Caldiserica
6	Actinobacteria	20	Chlamydiae
7	Verrucomicrobia	21	Chlorobi
8	Spirochaetes	22	Chrysiogenetes
9	Cyanobacteria	23	Crenarchaeota
10	Tenericutes	24	Fibrobacteres
11	Chloroflexi	25	Nitrospirae
12	Synergistetes	26	Planctomycetes
13	Euryarchaeota	27	Armatimonadetes
14	Thermotogae		

Table 2: 626 genera identified in the subgingival samples.

1	Abiotrophia	41	Allochromatium	81	Balneola	121	Catonella	161	Delftia
2	Acetobacterium	42	Amaricoccus	82	Bartonella	122	Caulobacter	162	Demequina
3	Acetohalobium	43	Aminiphilus	83	Bdellovibrio	123	Cellulomonas	163	Denitratisoma
4	Acholeplasma	44	Aminobacterium	84	Beggiatoa	124	Cellulophaga	164	Denitrobacter
5	Achromobacter	45	Ammonifex	85	Bellilinea	125	Cellvibrio	165	Dermacoccus
6	Acidaminobacter	46	Ammoniphilus	86	Bergeyella	126	Cerasicoccus	166	Dermatophilus
7	Acidaminococcus	47	Amphritea	87	Bifidobacterium	127	Cetobacterium	167	Desemzia
8	Acidianus	48	Amycolatopsis	88	Bizionia	128	Chelonobacter	168	Desulfitobacterium
9	Acidimicrobium	49	Anaerobacillus	89	Blautia	129	Chitinophaga	169	Desulfobacter
10	Acidiphilium	50	Anaerobranca	90	Borrelia	130	Chlorobaculum	170	Desulfobulbus
11	Acidisphaera	51	Anaerococcus	91	Brachybacterium	131	Chloroherpeton	171	Desulfofrigus
12	Acidithiobacillus	52	Anaerofilum	92	Brachyspira	132	Chondromyces	172	Desulfomicrobium
13	Acidovorax	53	Anaerolinea	93	Bradyrhizobium	133	Chromatium	173	Desulfomonile
14	Acinetobacter	54	Anaeromusa	94	Brenneria	134	Chromobacterium	174	Desulfonatronovibrio
15	Actinoallomurus	55	Anaeroplasma	95	Brevibacillus	135	Chroococcus	175	Desulfonatronum
16	Actinobacillus	56	Anaerovibrio	96	Brevibacterium	136	Chryseobacterium	176	Desulfonauticus
17	Actinobaculum	57	Ancylobacter	97	Brevundimonas	137	Clostridium	177	Desulfosarcina
18	Actinocatenispora	58	Aneurinibacillus	98	Brochothrix	138	Cohnella	178	Desulfosporosinus
19	Actinocorallia	59	Anoxybacillus	99	Bulleidia	139	Collimonas	179	Desulfotomaculum
20	Actinokineospora	60	Aquimarina	100	Burkholderia	140	Collinsella	180	Desulfovibrio
21	Actinomadura	61	Aquitalea	101	Butyricimonas	141	Colwellia	181	Desulfurella
22	Actinomyces	62	Arcanobacterium	102	Butyrivibrio	142	Comamonas	182	Desulfurispirillum
23	Actinoplanes	63	Archaeoglobus	103	Caldanaerobacter	143	Conchiformibius	183	Desulfurispora
24	Actinopolymorpha	64	Arcobacter	104	Caldicellulosiruptor	144	Coprococcus	184	Desulfurococcus
25	Actinopolyspora	65	Arenibacter	105	Caldilinea	145	Coraliomargarita	185	Desulfuromonas
26	Adlercreutzia	66	Arenimonas	106	Caldisericum	146	Coriobacterium	186	Desulfuromusa
27	Aequorivita	67	Armatimonas	107	Caldisphaera	147	Corynebacterium	187	Dethiobacter
28	Aerococcus	68	Arsenophonus	108	Caldithrix	148	Crocosphaera	188	Dethiosulfovibrio
29	Aeromicrobium	69	Arthrobacter	109	Caloramator	149	Cryptosporangium	189	Devosia
30	Aggregatibacter	70	Arthronema	110	Calothrix	150	Cupriavidus	190	Dialister
31	Agrobacterium	71	Asticcacaulis	111	Caminibacter	151	Curvibacter	191	Diaphorobacter
32	Agrococcus	72	Atopobium	112	Campylobacter	152	Cycloclasticus	192	Dichelobacter
33	Agromyces	73	Avibacterium	113	Candidatus	153	Cystobacter	193	Dietzia
34	Alcanivorax	74	Azoarcus	114	Candidatus Aquiluna	154	Dactylosporangium	194	Dokdonella
35	Alicycliphilus	75	Azohydromonas	115	Candidatus Scalindua	155	Dechloromonas	195	Dolichospermum
36	Alishewanella	76	Azomonas	116	Capnocytophaga	156	Deefgea	196	Dyadobacter
37	Alkalibacillus	77	Azorhizophilus	117	Carboxydocella	157	Deferribacter	197	Dysgonomonas
38	Alkalibacterium	78	Azospirillum	118	Cardiobacterium	158	Dehalobacterium	198	Ectothiorhodospira
39	Alkaliphilus	79	Bacillus	119	Carnobacterium	159	Dehalogenimonas	199	Edaphobacter
40	Allobaculum	80	Bacteroides	120	Catenulispora	160	Deinococcus	200	Eggerthella

201	Ehrlichia	241	Gillisia	281	Klebsiella	321	Maribacter	361	Moritella
202	Eikenella	242	Glaciecola	282	Knoellia	322	Maricaulis	362	Moryella
203	Elizabethkingia	243	Gloeotrichia	283	Kocuria	323	Marichromatium	363	Muricauda
204	Emticicia	244	Gluconobacter	284	Kosmotoga	324	Marinilactibacillus	364	Mycobacterium
205	Enhydrobacter	245	Glycomyces	285	Kouleothrix	325	Marinitoga	365	Mycoplasma
206	Enterobacter	246	Gordonia	286	Kribbella	326	Marinobacter	366	Myroides
207	Enterococcus	247	Gramella	287	Kurthia	327	Marinobacterium	367	Nannocystis
208	Entomoplasma	248	Granulicatella	288	Kushneria	328	Marinococcus	368	Natroniella
209	Erwinia	249	Granulicella	289	Labrys	329	Marinomonas	369	Natronincola
210	Erysipelothrix	250	Haemophilus	290	Laceyella	330	Marinospirillum	370	Nautilia
211	Erythrobacter	251	Hahella	291	Lachnospira	331	Megamonas	371	Negativicoccus
212	Euzebya	252	Halanaerobacter	292	Lactobacillus	332	Megasphaera	372	Neisseria
213	Exiguobacterium	253	Halanaerobium	293	Lactococcus	333	Meiothermus	373	Neorickettsia
214	Facklamia	254	Haliangium	294	Lampropedia	334	Melissococcus	374	Nesterenkonia
215	Faecalibacterium	255	Haloanella	295	Lautropia	335	Mesoplasma	375	Niabella
216	Ferrimicrobium	256	Halomonas	296	Legionella	336	Mesorhizobium	376	Nisaea
217	Ferrimonas	257	Helcococcus	297	Lentibacillus	337	Metallosphaera	377	Nitriliruptor
218	Fervidobacterium	258	Helicobacter	298	Lentzea	338	Methanobacterium	378	Nitrincola
219	Fibrobacter	259	Heliorestis	299	Leptolyngbya	339	Methanobrevibacter	379	Nitrobacter
220	Filifactor	260	Herbaspirillum	300	Leptospira	340	Methanocorpusculum	380	Nitrosococcus
221	Flammeovirga	261	Holdemania	301	Leptothrix	341	Methanogenium	381	Nitrospina
222	Flavisolibacter	262	Hydrocarboniphaga	302	Leptotrichia	342	Methylibium	382	Nocardia
223	Flavobacterium	263	Hydrogenophaga	303	Leucobacter	343	Methylobacillus	383	Nocardioides
224	Francisella	264	Hydrogenophilus	304	Leuconostoc	344	Methylobacterium	384	Nocardiopsis
225	Frankia	265	Hylemonella	305	Leucothrix	345	Methylocella	385	Nonomuraea
226	Friedmanniella	266	Hymenobacter	306	Lewinella	346	Methylomicrobium	386	Nostoc
227	Fructobacillus	267	Hyphomicrobium	307	Limnobacter	347	Methylonatrum	387	Novosphingobium
228	Fulvivirga	268	Hyphomonas	308	Limnohabitans	348	Methylophaga	388	Oceanisphaera
229	Fusibacter	269	Isoptericola	309	Listeria	349	Methylotenera	389	Ochrobactrum
230	Fusobacterium	270	Janibacter	310	Litoricola	350	Methyloversatilis	390	Odoribacter
231	Gallibacterium	271	Janthinobacterium	311	Longilinea	351	Microbacterium	391	Oenococcus
232	Gallionella	272	Jeotgalicoccus	312	Luteibacter	352	Microbulbifer	392	Oligella
233	Gemella	273	Jiangella	313	Luteimonas	353	Micrococcus	393	Olivibacter
234	Geobacillus	274	Johnsonella	314	Luteococcus	354	Microcystis	394	Olsenella
235	Geobacter	275	Jonesia	315	Luteolibacter	355	Micromonospora	395	Oribacterium
236	Georgenia	276	Kaistella	316	Lutimonas	356	Microvirgula	396	Oscillatoria
237	Geothrix	277	Kaistobacter	317	Lysinibacillus	357	Mitsuokella	397	Oscillochloris
238	Geotoga	278	Kineosporia	318	Macrococcus	358	Mogibacterium	398	Oscillospira
239	Geovibrio	279	Kingella	319	Magnetospirillum	359	Moorella	399	Oxalobacter
240	Giesbergeria	280	Kitasatospora	320	Mannheimia	360	Moraxella	400	Paenibacillus

401	Paenisporosarcina	441	Pontibacillus	481	Rhodovibrio	521	Shuttleworthia	561	Tenacibaculum	601	Trichococcus
402	Paludibacter	442	Pontibacter	482	Rhodovulum	522	Simplicispira	562	Tepidanaerobacter	602	Trichodesmium
403	Parabacteroides	443	Porphyromonas	483	Rickettsia	523	Singulisphaera	563	Tepidimicrobium	603	Tsukamurella
404	Paracoccus	444	Prauserella	484	Rickettsiella	524	Slackia	564	Tepidimonas	604	Turicibacter
405	Parapedobacter	445	Prevotella	485	Riemerella	525	Smithella	565	Tessaracoccus	605	Uliginosibacterium
406	Paraprevotella	446	Promicromonospora	486	Rikenella	526	Sneathia	566	Tetragenococcus	606	Ureibacillus
407	Parascardovia	447	Propionibacterium	487	Rivularia	527	Snowella	567	Tetrasphaera	607	Vagococcus
408	Pasteurella	448	Propionicimonas	488	Robiginitalea	528	Soehngenia	568	Thalassospira	608	Variovorax
409	Patulibacter	449	Propionigenium	489	Roseococcus	529	Sorangium	569	Thauera	609	Veillonella
410	Paucibacter	450	Propionispora	490	Roseomonas	530	Sphaerochaeta	570	Thermacetogenium	610	Vibrio
411	Pectinatus	451	Propionivibrio	491	Roseospira	531	Sphingobacterium	571	Thermicanus	611	Virgibacillus
412	Pediococcus	452	Providencia	492	Rothia	532	Sphingobium	572	Thermoactinomyces	612	Viridibacillus
413	Pedobacter	453	Pseudaminobacter	493	Rubritalea	533	Sphingomonas	573	Thermoanaerobacter	613	Vitreoscilla
414	Pedomicrobium	454	Pseudidiomarina	494	Rubrivivax	534	Spirosoma	574	Thermoanaerobacterium	614	Vogesella
415	Pelagicoccus	455	Pseudoalteromonas	495	Runella	535	Sporanaerobacter	575	Thermobacillus	615	Waddlia
416	Pelobacter	456	Pseudochrobactrum	496	Saccharomonospora	536	Sporichthya	576	Thermobaculum	616	Weissella
417	Pelomonas	457	Pseudoclavibacter	497	Saccharopolyspora	537	Sporolactobacillus	577	Thermococcus	617	Winogradskyella
418	Pelotomaculum	458	Pseudomonas	498	Saccharospirillum	538	Sporosarcina	578	Thermodesulfatator	618	Xanthobacter
419	Peptococcus	459	Pseudonocardia	499	Saccharothrix	539	Sporotomaculum	579	Thermodesulfovibrio	619	Xanthomonas
420	Peptoniphilus	460	Psychrobacter	500	Salegentibacter	540	Staphylococcus	580	Thermogemmatispora	620	Xenophilus
421	Peptostreptococcus	461	Psychroflexus	501	Salinicoccus	541	Stenotrophomonas	581	Thermomonas	621	Xylanimicrobium
422	Petrotoga	462	Psychromonas	502	Salinimicrobium	542	Stenoxybacter	582	Thermosipho	622	Yaniella
423	Phaeobacter	463	Pullulanibacillus	503	Salinispora	543	Steroidobacter	583	Thermovenabulum	623	Yersinia
424	Phascolarctobacterium	464	Pyramidobacter	504	Salinivibrio	544	Sterolibacterium	584	Thermus	624	Zhihengliuella
425	Phenylobacterium	465	Ralstonia	505	Salisaeta	545	Streptacidiphilus	585	Thioalkalimicrobium	625	Zhouia
426	Phormidium	466	Ramlibacter	506	Salmonella	546	Streptobacillus	586	Thioalkalivibrio	626	Zobellia
427	Photobacterium	467	Rarobacter	507	Sanguibacter	547	Streptococcus	587	Thiobacillus		
428	Photorhabdus	468	Rathayibacter	508	Scardovia	548	Streptomyces	588	Thiobacter		
429	Phycicoccus	469	Renibacterium	509	Sebaldella	549	Streptosporangium	589	Thiocapsa		
430	Phyllobacterium	470	Rheinheimera	510	Sedimentibacter	550	Succinivibrio	590	Thiohalorhabdus		
431	Pilimelia	471	Rhizobium	511	Sediminibacillus	551	Sulfobacillus	591	Thiomicrospira		
432	Pimelobacter	472	Rhodanobacter	512	Sediminibacterium	552	Sulfurimonas	592	Thiomonas		
433	Piscirickettsia	473	Rhodobacter	513	Segetibacter	553	Sulfurospirillum	593	Thioploca		
434	Planctomyces	474	Rhodococcus	514	Selenomonas	554	Sutterella	594	Thiorhodococcus		
435	Planifilum	475	Rhodocyclus	515	Serinicoccus	555	Symbiobacterium	595	Thiorhodospira		
436	Planococcus	476	Rhodoferax	516	Serratia	556	Symploca	596	Thiothrix		
437	Planomicrobium	477	Rhodoplanes	517	Sharpea	557	Syntrophobacter	597	Tindallia		
438	Polaribacter	478	Rhodospirillum	518	Shewanella	558	Syntrophomonas	598	Tolumonas		
439	Polaromonas	479	Rhodothalassium	519	Shimazuella	559	Tannerella	599	Trabulsiella		
440	Polynucleobacter	480	Rhodothermus	520	Shinella	560	Telmatospirillum	600	Treponema		

Table 3: Health associated microbiome

1	Abiotrophia defectiva	51	Alkalibacillus salilacus	101	Bacillus olivae
2	Acetobacterium tundrae	52	Alkalibacterium subtropicum	102	Bacillus psychrosaccharolyticus
3	Acholeplasma ales	53	Alkaliphilus crotonatoxidans	103	Bacillus velezensis
4	Acholeplasma cavigenitalium	54	Alkaliphilus metalliredigens	104	Bacteroides denticanum
5	Acholeplasma palmae	55	Alkaliphilus peptidifermentans	105	Bacteroides gallinarum
6	Acholeplasma parvum	56	Allobaculum stercoricanis	106	Bacteroides graminisolvens
7	Achromobacter ruhlandii	57	Allochromatium palmeri	107	Bacteroides heparinolyticus
8	Acidaminobacter hydrogenoformans	58	Amaricoccus kaplicensis	108	Bacteroides oleiciplenus
9	Acidaminococcus intestini	59	Aminiphilus circumscriptus	109	Bacteroides paurosaccharolyticus
10	Acidiphilium symbioticum	60	Ammonifex thiophilus	110	Bacteroides propionicifaciens
11	Acidovorax temperans	61	Ammoniphilus oxalivorans	111	Bacteroides rodentium
12	Acinetobacter antiviralis	62	Amycolatopsis helveola	112	Bacteroides salanitronis
13	Acinetobacter gerneri	63	Amycolatopsis jejuensis	113	Bacteroides sartorii
14	Acinetobacter guillouiae	64	Amycolatopsis methanolica	114	Bacteroides stercorirosoris
15	Actinobacillus parahaemolyticus	65	Amycolatopsis palatopharyngis	115	Bacteroides xylanisolvens
16	Actinobacillus pleuropneumoniae	66	Anaerobacillus alkalilacustre	116	Bacteroides zoogleoformans
17	Actinobacillus porcinus	67	Anaerobranca zavarzinii	117	Balneola vulgaris
18	Actinobacillus rossii	68	Anaerococcus octavius	118	Bdellovibrio exovorus
19	Actinobaculum massiliense	69	Anaerolinea thermolimosa	119	Bellilinea caldifistulae
20	Actinobaculum suis	70	Anaeromusa acidaminophila	120	Bergeyella zoohelcum
21	Actinocorallia cavernae	71	Anaeroplasma abactoclasticum	121	Bifidobacterium bombi
22	Actinocorallia herbida	72	Anaerovibrio lipolyticus	122	Bifidobacterium indicum
23	Actinokineospora inagensis	73	Anoxybacillus eryuanensis	123	Bifidobacterium scardovii
24	Actinomadura maheshkhaliensis	74	Aquimarina macrocephali	124	Bifidobacterium subtile
25	Actinomadura verrucosospora	75	Aquitalea denitrificans	125	Blautia coccoides
26	Actinomyces cardiffensis	76	Arcanobacterium bernardiae	126	Blautia wexlerae
27	Actinomyces coleocanis	77	Arcanobacterium haemolyticum	127	Brachyspira ibaraki
28	Actinomyces europaeus	78	Arcobacter marinus	128	Brevibacillus ginsengisoli
29	Actinomyces georgiae	79	Arcobacter skirrowii	129	Brevibacterium samyangense
30	Actinomyces lingnae	80	Arcobacter thereius	130	Brevundimonas olei
31	Actinomyces meyeri	81	Arenibacter certesii	131	Brochothrix thermosphacta
32	Actinomyces naturae	82	Arenibacter troitsensis	132	Bulleidia extructa
33	Actinomyces odontolyticus	83	Arthrobacter psychrochitiniphilus	133	Bulleidia moorei
34	Actinomyces suimastitidis	84	Arthrobacter uratoxydans	134	Burkholderia brasilensis
35	Actinomyces turicensis	85	Arthronema africanum	135	Burkholderia caledonica
36	Actinomyces vaccimaxillae	86	Atopobium fossor	136	Burkholderia fungorum
37	Actinoplanes digitatis	87	Atopobium parvulum	137	Burkholderia graminis
38	Actinopolymorpha rutila	88	Atopobium rimae	138	Burkholderia phenazinium
39	Aequorivita crocea	89	Avibacterium avium	139	Burkholderia phenoliruptrix
40	Aequorivita lipolytica	90	Azomonas insignis	140	Burkholderia ubonensis
41	Aerococcus christensenii	91	Azomonas macrocytogenes	141	Butyricimonas synergistica
42	Aeromicrobium ponti	92	Azorhizophilus paspali	142	Butyricimonas virosa
43	Aggregatibacter aphrophilus	93	Azospirillum palatum	143	Butyrivibrio proteoclasticus
44	Aggregatibacter segnis	94	Bacillus alcaliinulinus	144	Caldanaerobacter hydrothermalis
45	Agrococcus versicolor	95	Bacillus anthracis	145	Caldicellulosiruptor bescii
46	Agromyces hippuratus	96	Bacillus deserti	146	Caldilinea tarbellica
47	Agromyces mediolanus	97	Bacillus ferrariarum	147	Caloramator indicus
48	Agromyces rhizospherae	98	Bacillus horneckiae	148	Caloramator mitchellensis
49	Agromyces salentinus	99	Bacillus isabeliae	149	Caloramator uzoniensis
50	Agromyces succinolyticus	100	Bacillus mucilaginosus	150	Caloramator viterbiensis

151	Calothrix brevissima	201	Chromatium weissei	251	Corynebacterium jeikeium
152	Calothrix parietina	202	Chromobacterium haemolyticum	252	Corynebacterium kutscheri
153	Campylobacter canadensis	203	Chromobacterium piscinae	253	Corynebacterium matruchotii
154	Campylobacter concisus	204	Chromobacterium pseudoviolaceum	254	Corynebacterium mustelae
155	Campylobacter curvus	205	Chromobacterium subtsugae	255	Corynebacterium nuruki
156	Campylobacter faecalis	206	Chroococcus minutus	256	Corynebacterium resistens
157	Campylobacter fetus	207	Chryseobacterium caeni	257	Corynebacterium ulceribovis
158	Campylobacter gracilis	208	Chryseobacterium culicis	258	Cupriavidus laharis
159	Campylobacter insulaenigrae	209	Chryseobacterium daecheongense	259	Cupriavidus oxalaticus
160	Campylobacter mucosalis	210	Chryseobacterium greenlandense	260	Cupriavidus pinatubonensis
161	Campylobacter rectus	211	Chryseobacterium hungaricum	261	Curvibacter gracilis
162	Campylobacter showae	212	Chryseobacterium isbiliense	262	Cycloclasticus oligotrophus
163	Campylobacter subantarcticus	213	Chryseobacterium joostei	263	Dactylosporangium fulvum
164	Campylobacter volucris	214	Chryseobacterium oranimense	264	Dactylosporangium maewongense
165	Candidatus Amoebophilus asiaticus	215	Chryseobacterium taichungense	265	Dechloromonas hortensis
166	Candidatus Azobacteroides pseudotrichonymphae	216	Clostridium acidisoli	266	Deferribacter autotrophicus
167	Candidatus Blochmannia rufipes	217	Clostridium aestuarii	267	Deinococcus yavapaiensis
168	Candidatus Contubernalis alkalaceticum	218	Clostridium alkalicellulosi	268	Delftia lacustris
169	Candidatus Endobugula glebosa	219	Clostridium cadaveris	269	Delftia tsuruhatensis
170	Candidatus Liberibacter africanus	220	Clostridium caenicola	270	Demequina aurantiaca
171	Candidatus Phlomobacter fragariae	221	Clostridium caliptrosporum	271	Denitratisoma oestradiolicum
172	Candidatus Phytoplasma fragariae	222	Clostridium fallax	272	Dermacoccus barathri
173	Candidatus Phytoplasma phoenicium	223	Clostridium frigoris	273	Desulfitobacterium metallireducens
174	Candidatus Phytoplasma pini	224	Clostridium grantii	274	Desulfofrigus oceanense
175	Candidatus Phytoplasma prunorum	225	Clostridium histolyticum	275	Desulfomonile tiedjei
176	Candidatus Rhabdochlamydia crassificans	226	Clostridium homopropionicum	276	Desulfonatronum cooperativum
177	Candidatus Tammella caduceiae	227	Clostridium malenominatum	277	Desulfonatronum thiosulfatophilum
178	Capnocytophaga canimorsus	228	Clostridium proteolyticum	278	Desulfonauticus autotrophicus
179	Capnocytophaga cynodegmi	229	Clostridium proteolyticus	279	Desulfosporosinus hippei
180	Capnocytophaga gingivalis	230	Clostridium straminisolvens	280	Desulfosporosinus lacus
181	Capnocytophaga granulosa	231	Clostridium sulfidigenes	281	Desulfotomaculum australicum
182	Capnocytophaga haemolytica	232	Clostridium taeniosporum	282	Desulfotomaculum halophilum
183	Capnocytophaga leadbetteri	233	Clostridium tagluense	283	Desulfotomaculum indicum
184	Capnocytophaga ochracea	234	Clostridium tepidiprofundi	284	Desulfotomaculum thermoacetoxidans
185	Carboxydocella ferrireducens	235	Clostridium thermoalcaliphilum	285	Desulfovibrio caledoniensis
186	Cardiobacterium hominis	236	Clostridium thermosuccinogenes	286	Desulfovibrio desulfuricans
187	Cardiobacterium valvarum	237	Clostridium tunisiense	287	Desulfovibrio fairfieldensis
188	Carnobacterium inhibens	238	Clostridium vincentii	288	Desulfovibrio profundus
189	Catenulispora yoronensis	239	Cohnella damuensis	289	Desulfovibrio psychrotolerans
190	Catonella morbi	240	Cohnella fontinalis	290	Desulfovibrio simplex
191	Cellulomonas gelida	241	Cohnella hongkongensis	291	Desulfovibrio tunisiensis
192	Cellulophaga fucicola	242	Cohnella laeviribosi	292	Desulfurispirillum alkaliphilum
193	Cellvibrio mixtus	243	Collimonas pratensis	293	Desulfurispora thermophila
194	Cerasicoccus arenae	244	Comamonas koreensis	294	Desulfuromonas acetoxidans
195	Cetobacterium ceti	245	Conchiformibius kuhniae	295	Desulfuromonas svalbardensis
196	Chelonobacter oris	246	Conchiformibius steedae	296	Dialister invisus
197	Chitinophaga soli	247	Coraliomargarita akajimensis	297	Dialister micraerophilus
198	Chlorobaculum limnaeum	248	Corynebacterium atypicum	298	Dichelobacter nodosus
199	Chloroherpeton thalassium	249	Corynebacterium doosanense	299	Dokdonella fugitiva
200	Chondromyces pediculatus	250	Corynebacterium durum	300	Dolichospermum curvum

301	Dyadobacter hamtensis	351	Gemella haemolysans	401	Kingella oralis
302	Dysgonomonas capnocytophagoides	352	Gemella morbillorum	402	Kitasatospora terrestris
303	Dysgonomonas hofstadii	353	Gemella sanguinis	403	Kocuria rosea
304	Dysgonomonas wimpennyi	354	Genus_Spieces_list	404	Kosmotoga arenicorallina
305	Ectothiorhodospira haloalkaliphila	355	Geobacillus thermoglucosidans	405	Kosmotoga olearia
306	Edaphobacter modestus	356	Geothrix fermentans	406	Kouleothrix aurantiaca
307	Eggerthella sinensis	357	Geotoga petraea	407	Kribbella ginsengisoli
308	Ehrlichia ovina	358	Gillisia limnaea	408	Kurthia sibirica
309	Eikenella corrodens	359	Gillisia sandarakina	409	Kushneria aurantia
310	Elizabethkingia anophelis	360	Glaciecola nitratireducens	410	Kushneria indalinina
311	Elizabethkingia meningoseptica	361	Gluconobacter krungthepensis	411	Laceyella putida
312	Emticicia oligotrophica	362	Gluconobacter morbifer	412	Lachnospira pectinoschiza
313	Enterococcus gilvus	363	Gordonia hydrophobica	413	Lactobacillus apis
314	Entomoplasma somnilux	364	Gramella forsetii	414	Lactobacillus equi
315	Erysipelothrix inopinata	365	Gramella marina	415	Lactobacillus hayakitensis
316	Erysipelothrix muris	366	Granulicatella adiacens	416	Lactobacillus intermedius
317	Erythrobacter aquimaris	367	Granulicatella elegans	417	Lactobacillus senmaizukei
318	Euzebya tangerina	368	Granulicella tundricola	418	Lactobacillus siliginis
319	Exiguobacterium soli	369	Haemophilus haemolyticus	419	Lactobacillus tucceti
320	Facklamia hominis	370	Haemophilus parainfluenzae	420	Lactococcus fujiensis
321	Facklamia languida	371	Haemophilus quentini	421	Lautropia mirabilis
322	Facklamia tabacinasalis	372	Hahella antarctica	422	Legionella sainthelensi
323	Ferrimicrobium acidiphilum	373	Halanaerobacter chitinivorans	423	Legionella shakespearei
324	Fibrobacter intestinalis	374	Halanaerobium alcaliphilum	424	Lentibacillus kapialis
325	Filifactor alocis	375	Halanaerobium fermentans	425	Lentibacillus salinarum
326	Filifactor villosus	376	Halomonas almeriensis	426	Leptolyngbya laminosa
327	Flammeovirga aprica	377	Halomonas johnsoniae	427	Leptospira licerasiae
328	Flammeovirga pacifica	378	Halomonas neptunia	428	Leptothrix discophora
329	Flavisolibacter ginsengisoli	379	Halomonas sabkhae	429	Leptotrichia buccalis
330	Flavobacterium antarcticum	380	Helcococcus ovis	430	Leptotrichia goodfellowii
331	Flavobacterium croceum	381	Helcococcus sueciensis	431	Leptotrichia hofstadii
332	Flavobacterium cucumis	382	Helicobacter baculiformis	432	Leptotrichia shahii
333	Flavobacterium denitrificans	383	Helicobacter mastomyrinus	433	Leptotrichia trevisanii
334	Flavobacterium filum	384	Helicobacter suncus	434	Leptotrichia wadei
335	Flavobacterium saliperosum	385	Herbaspirillum aquaticum	435	Leucothrix mucor
336	Flavobacterium swingsii	386	Herbaspirillum magnetovibrio	436	Lewinella lutea
337	Flavobacterium terrigena	387	Hydrocarboniphaga daqingensis	437	Lewinella marina
338	Francisella hispaniensis	388	Hydrogenophaga defluvii	438	Limnobacter litoralis
339	Frankia alni	389	Hydrogenophilus denitrificans	439	Listeria innocua
340	Fructobacillus pseudoficulneus	390	Hydrogenophilus halorhabdus	440	Litoricola lipolytica
341	Fulvivirga kasyanovii	391	Hydrogenophilus hirschii	441	Longilinea arvoryzae
342	Fusobacterium canifelinum	392	Hymenobacter gelipurpurascens	442	Luteibacter anthropi
343	Fusobacterium gonidiaformans	393	Hymenobacter ocellatus	443	Luteimonas terricola
344	Fusobacterium naviforme	394	Hymenobacter rigui	444	Luteococcus peritonei
345	Fusobacterium nucleatum	395	Hyphomicrobium aestuarii	445	Luteolibacter algae
346	Fusobacterium periodonticum	396	Janthinobacterium agaricidamnosum	446	Lysinibacillus parviboronicapiens
347	Fusobacterium simiae	397	Jeotgalicoccus nanhaiensis	447	Macrococcus bovicus
348	Gallibacterium melopsittaci	398	Johnsonella ignava	448	Magnetospirillum bellicus
349	Gallionella ferruginea	399	Jonesia quinghaiensis	449	Mannheimia caviae
350	Gemella cunicula	400	Kaistobacter terrae	450	Mannheimia granulomatis

451	Maricaulis indicus	501	Mycobacterium chitae	551	Paenibacillus contaminans
452	Marichromatium gracile	502	Mycobacterium diernhoferi	552	Paenibacillus darangshiensis
453	Marinitoga camini	503	Mycobacterium engbaekii	553	Paenibacillus ourofinensis
454	Marinitoga hydrogenitolerans	504	Mycobacterium lepromatosis	554	Paenibacillus pinihumi
455	Marinobacter arcticus	505	Mycobacterium novocastrense	555	Parabacteroides goldsteinii
456	Marinobacter salicampi	506	Mycobacterium pinnipedii	556	Parabacteroides gordonii
457	Marinobacter squalenivorans	507	Mycobacterium senuense	557	Parabacteroides johnsonii
458	Marinobacter szutsaonensis	508	Mycoplasma agassizii	558	Paracoccus seriniphilus
459	Marinobacterium sediminicola	509	Mycoplasma corogypsi	559	Parapedobacter koreensis
460	Marinomonas basaltis	510	Mycoplasma edwardii	560	Paraprevotella clara
461	Marinomonas brasiliensis	511	Mycoplasma fastidiosum	561	Pasteurella pneumotropica
462	Marinomonas pontica	512	Mycoplasma haemominutum	562	Patulibacter americanus
463	Megamonas funiformis	513	Mycoplasma iguanae	563	Pectinatus cerevisiiphilus
464	Megasphaera geminatus	514	Mycoplasma insons	564	Pediococcus argentinicus
465	Megasphaera hominis	515	Mycoplasma lipophilum	565	Pedobacter daejeonensis
466	Megasphaera micronuciformis	516	Mycoplasma moatsii	566	Pedobacter himalayensis
467	Megasphaera paucivorans	517	Mycoplasma salivarium	567	Pedobacter kwangyangensis
468	Megasphaera sueciensis	518	Mycoplasma timone	568	Pedomicrobium ferrugineum
469	Meiothermus granaticius	519	Mycoplasma verecundum	569	Pelagicoccus croceus
470	Mesoplasma entomophilum	520	Myroides injenensis	570	Pelotomaculum isophthalicicum
471	Metallosphaera hakonensis	521	Myroides odoratus	571	Pelotomaculum terephthalicicum
472	Methanocorpusculum parvum	522	Myroides profundi	572	Peptococcus niger
473	Methylobacillus flagellatus	523	Natroniella acetigena	573	Peptoniphilus coxii
474	Methylobacillus glycogenes	524	Negativicoccus succinicivorans	574	Peptoniphilus gorbachii
475	Methylomicrobium pelagicum	525	Neisseria cinerea	575	Peptoniphilus indolicus
476	Methylonatrum kenyense	526	Neisseria elongata	576	Peptoniphilus ivorii
477	Methylophaga alcalica	527	Neisseria flavescens	577	Peptoniphilus methioninivorax
478	Methylophaga lonarensis	528	Neisseria lactamica	578	Peptoniphilus olsenii
479	Methylotenera versatilis	529	Neisseria mucosa	579	Phascolarctobacterium succinatutens
480	Methyloversatilis universalis	530	Neisseria polysaccharea	580	Planctomyces maris
481	Microbacterium halophilum	531	Neisseria subflava	581	Planifilum fimeticola
482	Microbacterium profundi	532	Neisseria weaveri	582	Planococcus columbae
483	Microbacterium xinjiangensis	533	Neorickettsia helminthoeca	583	Planococcus maritimus
484	Microbulbifer donghaiensis	534	Nisaea nitritireducens	584	Planomicrobium alkanoclasticum
485	Micrococcus yunnanensis	535	Nitrincola lacisaponensis	585	Planomicrobium flavidum
486	Microcystis panniformis	536	Nitrobacter hamburgensis	586	Planomicrobium stackebrandtii
487	Micromonospora aquatica	537	Nitrospina gracilis	587	Polaribacter butkevichii
488	Micromonospora fulvoviolacea	538	Nonomuraea asiatica	588	Polaribacter dokdonensis
489	Micromonospora rifamycinica	539	Nostoc piscinale	589	Polaromonas vacuolata
490	Microvirgula aerodenitrificans	540	Ochrobactrum thiophenivorans	590	Polynucleobacter rarus
491	Mitsuokella jalaludinii	541	Odoribacter denticanis	591	Pontibacillus chungwhensis
492	Mogibacterium neglectum	542	Odoribacter laneus	592	Pontibacillus halophilus
493	Mogibacterium timidum	543	Oligella ureolytica	593	Pontibacillus marinus
494	Mogibacterium vescum	544	Olivibacter soli	594	Pontibacter niistensis
495	Moorella mulderi	545	Olsenella uli	595	Porphyromonas asaccharolytica
496	Moraxella caviae	546	Oribacterium sinus	596	Porphyromonas cangingivalis
497	Moritella japonica	547	Oscillatoria corallinae	597	Porphyromonas canis
498	Moryella indoligenes	548	Oscillospira eae	598	Porphyromonas canoris
499	Muricauda lutimaris	549	Oscillospira guilliermondii	599	Porphyromonas cansulci
500	Mycobacterium austroafricanum	550	Oxalobacter vibrioformis	600	Porphyromonas catoniae

601	Porphyromonas circumdentaria	651	Pseudomonas citronellolis	701	Rhodovibrio sodomensis
602	Porphyromonas endodontalis	652	Pseudomonas clemancea	702	Rickettsia hulinii
603	Porphyromonas gingivalis	653	Pseudomonas coronafaciens	703	Rickettsia monacensis
604	Porphyromonas gulae	654	Pseudomonas fluorescens	704	Rikenella microfusus
605	Porphyromonas macacae	655	Pseudomonas fuscovaginae	705	Robiginitalea biformata
606	Prevotella albensis	656	Pseudomonas guineae	706	Roseococcus thiosulfatophilus
607	Prevotella amnii	657	Pseudomonas jinjuensis	707	Roseomonas massiliensis
608	Prevotella aurantiaca	658	Pseudomonas koreensis	708	Roseomonas terpenica
609	Prevotella bergensis	659	Pseudomonas lundensis	709	Roseomonas terrae
610	Prevotella buccae	660	Pseudomonas mandelii	710	Roseospira mediosalina
611	Prevotella buccalis	661	Pseudomonas mediterranea	711	Rothia aeria
612	Prevotella dentalis	662	Pseudomonas mendocina	712	Rothia dentocariosa
613	Prevotella dentasini	663	Pseudomonas metavorans	713	Runella limosa
614	Prevotella enoeca	664	Pseudomonas monteilii	714	Saccharomonospora thermoviridis
615	Prevotella histicola	665	Pseudomonas moraviensis	715	Saccharopolyspora cebuensis
616	Prevotella loescheii	666	Pseudomonas mucidolens	716	Saccharopolyspora flava
617	Prevotella maculosa	667	Pseudomonas nitroreducens	717	Salinicoccus iranensis
618	Prevotella marshii	668	Pseudomonas orientalis	718	Salinimicrobium terrae
619	Prevotella melaninogenica	669	Pseudomonas oryzihabitans	719	Salinispora tropica
620	Prevotella micans	670	Pseudomonas otitidis	720	Sanguibacter suarezii
621	Prevotella multiformis	671	Pseudomonas panacis	721	Sebaldella termitidis
622	Prevotella multisaccharivorax	672	Pseudomonas panipatensis	722	Sedimentibacter hongkongensis
623	Prevotella nanceiensis	673	Pseudomonas pavonaceae	723	Sedimentibacter hydroxybenzoicus
624	Prevotella nigrescens	674	Pseudomonas plecoglossicida	724	Segetibacter aerophilus
625	Prevotella oralis	675	Pseudomonas poae	725	Selenomonas artemidis
626	Prevotella oris	676	Pseudomonas pseudoalcaligenes	726	Selenomonas dianae
627	Prevotella oulorum	677	Pseudomonas putida	727	Selenomonas flueggei
628	Prevotella pallens	678	Pseudomonas resinovorans	728	Selenomonas infelix
629	Prevotella paludivivens	679	Pseudomonas rhodesiae	729	Selenomonas noxia
630	Prevotella pleuritidis	680	Pseudomonas stutzeri	730	Serinicoccus chungangensis
631	Prevotella saccharolytica	681	Pseudomonas teessidea	731	Serratia ureilytica
632	Prevotella salivae	682	Pseudomonas thermotolerans	732	Shewanella pneumatophori
633	Prevotella shahii	683	Pseudomonas tolaasii	733	Shinella fusca
634	Prevotella tannerae	684	Pseudomonas tremae	734	Shinella yambaruensis
635	Prevotella timonensis	685	Pseudomonas tropicalis	735	Slackia exigua
636	Prevotella veroralis	686	Pseudomonas vancouverensis	736	Slackia piriformis
637	Propionibacterium humerusii	687	Pseudomonas xanthomarina	737	Sneathia sanguinegens
638	Propionibacterium microaerophilum	688	Psychrobacter glacialis	738	Snowella rosea
639	Propionigenium modestum	689	Psychrobacter halophilus	739	Soehngenia saccharolytica
640	Propionispora hippei	690	Psychrobacter phenylpyruvicus	740	Sphaerochaeta coccoides
641	Propionivibrio dicarboxylicus	691	Psychroflexus gondwanensis	741	Sphingobacterium bambusae
642	Propionivibrio limicola	692	Ralstonia detusculanense	742	Sphingobacterium shayense
643	Propionivibrio pelophilus	693	Ralstonia insidiosa	743	Sphingomonas roseiflava
644	Pseudaminobacter defluvii	694	Rarobacter faecitabidus	744	Sphingomonas soli
645	Pseudoclavibacter helvolus	695	Rathayibacter rathayi	745	Sporolactobacillus putidus
646	Pseudomonas aeruginosa	696	Rhodanobacter thiooxydans	746	Sporosarcina pasteurii
647	Pseudomonas alcaligenes	697	Rhodobacter apigmentum	747	Sporotomaculum syntrophicum
648	Pseudomonas amygdali	698	Rhodococcus percolatus	748	Staphylococcus intermedius
649	Pseudomonas anguilliseptica	699	Rhodocyclus purpureus	749	Stenotrophomonas nitritireducens
650	Pseudomonas caricapapayae	700	Rhodothermus clarus	750	Steroidobacter denitrificans

751	Sterolibacterium denitrificans	801	Sulfobacillus yellowstonensis	851	Treponema paraluiscuniculi
752	Streptacidiphilus griseus	802	Sulfurimonas denitrificans	852	Treponema porcinum
753	Streptococcus agalactiae	803	Sulfurimonas paralvinellae	853	Treponema putidum
754	Streptococcus alactolyticus	804	Sutterella sanguinus	854	Treponema socranskii
755	Streptococcus anginosus	805	Symbiobacterium toebii	855	Treponema succinifaciens
756	Streptococcus australis	806	Symploca atlantica	856	Trichococcus flocculiformis
757	Streptococcus bovis	807	Syntrophomonas bryantii	857	Tsukamurella paurometabola
758	Streptococcus castoreus	808	Syntrophomonas cellicola	858	Turicibacter sanguinis
759	Streptococcus cristatus	809	Syntrophomonas sapovorans	859	Uliginosibacterium gangwonense
760	Streptococcus dentapri	810	Tannerella forsythia	860	Ureibacillus terrenus
761	Streptococcus dentirousetti	811	Telmatospirillum siberiense	861	Vagococcus teuberi
762	Streptococcus fryi	812	Tenacibaculum japonica	862	Variovorax boronicumulans
763	Streptococcus gallinaceus	813	Tenacibaculum litoreum	863	Veillonella atypica
764	Streptococcus gordonii	814	Tepidanaerobacter syntrophicus	864	Veillonella criceti
765	Streptococcus halichoeri	815	Tetragenococcus doogicus	865	Veillonella denticariosi
766	Streptococcus hyointestinalis	816	Tetragenococcus koreensis	866	Veillonella dispar
767	Streptococcus infantis	817	Tetragenococcus muriaticus	867	Veillonella montpellierensis
768	Streptococcus intermedius	818	Tetrasphaera vanveenii	868	Veillonella parvula
769	Streptococcus lactarius	819	Thalassospira tepidiphila	869	Virgibacillus salexigens
770	Streptococcus marimammalium	820	Thalassospira xianhensis	870	Viridibacillus neidei
771	Streptococcus milleri	821	Thauera aromatica	871	Vitreoscilla stercoraria
772	Streptococcus mitis	822	Thauera mechernichensis	872	Vogesella perlucida
773	Streptococcus oligofermentans	823	Thermacetogenium phaeum	873	Weissella hanii
774	Streptococcus oralis	824	Thermoactinomyces intermedius	874	Weissella salipiscis
775	Streptococcus orisratti	825	Thermoanaerobacter acetoethylicus	875	Weissella soli
776	Streptococcus parasanguinis	826	Thermoanaerobacter sulfurigignens	876	Xanthobacter polyaromaticivorans
777	Streptococcus peroris	827	Thermoanaerobacterium islandicum	877	Xanthomonas oryzae
778	Streptococcus pluranimalium	828	Thermobaculum terrenum	878	Xylanimicrobium pachnodae
779	Streptococcus plurextorum	829	Thermodesulfatator atlanticus	879	Zhihengliuella salsuginis
780	Streptococcus pseudopneumoniae	830	Thermodesulfovibrio thiophilus	880	Zhouia amylolytica
781	Streptococcus sanguinis	831	Thermogemmatispora foliorum	881	Zobellia laminariae
782	Streptococcus thermophilus	832	Thermogemmatispora onikobensis		
783	Streptococcus tigurinus	833	Thermomonas dokdonensis		
784	Streptococcus troglodytae	834	Thermomonas haemolytica		
785	Streptococcus uberis	835	Thermovenabulum ferriorganovorum		
786	Streptococcus urinalis	836	Thermus rehai		
787	Streptococcus ursoris	837	Thioalkalimicrobium sibiricum		
788	Streptococcus vestibularis	838	Thiobacillus sajanensis		
789	Streptomyces argillaceus	839	Thiobacillus thiophilus		
790	Streptomyces auratus	840	Thiohalorhabdus denitrificans		
791	Streptomyces coriofaciens	841	Thiomonas perometabolis		
792	Streptomyces danangensis	842	Thiomonas thermosulfata		
793	Streptomyces lazureus	843	Thiorhodococcus mannitoliphagus		
794	Streptomyces matensis	844	Thiorhodococcus pfennigii		
795	Streptomyces nanchangensis	845	Thiothrix fructosivorans		
796	Streptomyces qinlingensis	846	Thiothrix ramosa		
797	Streptomyces roseogilvus	847	Tolumonas auensis		
798	Streptomyces vitaminophilus	848	Treponema lecithinolyticum		
799	Streptosporangium purpuratum	849	Treponema maltophilum		
800	Sulfobacillus sibiricus	850	Treponema medium		

Table 4: Disease associated microbiome

1	Abiotrophia defectiva	51	Alcanivorax indicus	101	Bacillus shandongensis
2	Acetobacterium fimetarium	52	Alkalibacillus salilacus	102	Bacteroides denticanum
3	Acholeplasma ales	53	Alkalibacterium subtropicum	103	Bacteroides gallinarum
4	Acholeplasma cavigenitalium	54	Alkaliphilus crotonatoxidans	104	Bacteroides graminisolvens
5	Acholeplasma palmae	55	Alkaliphilus metalliredigens	105	Bacteroides heparinolyticus
6	Acholeplasma parvum	56	Alkaliphilus peptidifermentans	106	Bacteroides nordii
7	Acidaminobacter hydrogenoformans	57	Alkaliphilus transvaalensis	107	Bacteroides oleiciplenus
8	Acidaminococcus intestini	58	Allobaculum stercoricanis	108	Bacteroides paurosaccharolyticus
9	Acinetobacter antiviralis	59	Aminiphilus circumscriptus	109	Bacteroides rodentium
10	Acinetobacter indicus	60	Ammonifex thiophilus	110	Bacteroides salanitronis
11	Acinetobacter tjernbergiae	61	Amycolatopsis helveola	111	Bacteroides sartorii
12	Acinetobacter xiamenensis	62	Amycolatopsis jejuensis	112	Bacteroides stercorirosoris
13	Actinoallomurus luridus	63	Amycolatopsis methanolica	113	Bacteroides xylanisolvens
14	Actinobacillus capsulatus	64	Amycolatopsis palatopharyngis	114	Bacteroides zoogleoformans
15	Actinobacillus parahaemolyticus	65	Anaerobacillus alkalilacustre	115	Bdellovibrio exovorus
16	Actinobacillus pleuropneumoniae	66	Anaerobranca zavarzinii	116	Bellilinea caldifistulae
17	Actinobacillus porcinus	67	Anaerococcus octavius	117	Bergeyella zoohelcum
18	Actinobacillus rossii	68	Anaerolinea thermolimosa	118	Bifidobacterium bombi
19	Actinobaculum massiliense	69	Anaeromusa acidaminophila	119	Bifidobacterium dentium
20	Actinobaculum suis	70	Anaerovibrio lipolyticus	120	Bifidobacterium gallinarum
21	Actinocorallia cavernae	71	Anoxybacillus amylolyticus	121	Bifidobacterium indicum
22	Actinocorallia herbida	72	Aquimarina macrocephali	122	Bifidobacterium scardovii
23	Actinokineospora inagensis	73	Aquitalea denitrificans	123	Bifidobacterium subtile
24	Actinomadura maheshkhaliensis	74	Arcanobacterium bernardiae	124	Blautia coccoides
25	Actinomadura verrucosospora	75	Archaeoglobus lithotrophicus	125	Blautia hansenii
26	Actinomyces canis	76	Arcobacter marinus	126	Blautia wexlerae
27	Actinomyces cardiffensis	77	Arcobacter skirrowii	127	Bradyrhizobium pachyrhizi
28	Actinomyces europaeus	78	Arcobacter thereius	128	Brenneria salicis
29	Actinomyces georgiae	79	Arenibacter certesii	129	Brevibacillus ginsengisoli
30	Actinomyces lingnae	80	Arenibacter troitsensis	130	Brevibacterium album
31	Actinomyces meyeri	81	Arenimonas malthae	131	Brochothrix thermosphacta
32	Actinomyces naturae	82	Arthrobacter psychrochitiniphilus	132	Bulleidia extructa
33	Actinomyces neuii	83	Arthrobacter uratoxydans	133	Bulleidia moorei
34	Actinomyces odontolyticus	84	Arthronema africanum	134	Burkholderia fungorum
35	Actinomyces suimastitidis	85	Asticcacaulis taihuensis	135	Burkholderia phenoliruptrix
36	Actinomyces turicensis	86	Atopobium fossor	136	Burkholderia ubonensis
37	Actinomyces vaccimaxillae	87	Atopobium minutum	137	Butyricimonas synergistica
38	Actinoplanes digitatis	88	Atopobium parvulum	138	Butyricimonas virosa
39	Actinopolymorpha rutila	89	Atopobium rimae	139	Butyrivibrio proteoclasticus
40	Aequorivita crocea	90	Avibacterium avium	140	Caldanaerobacter hydrothermalis
41	Aerococcus christensenii	91	Azospirillum palatum	141	Caldicellulosiruptor bescii
42	Aeromicrobium ponti	92	Bacillus alcaliinulinus	142	Caldilinea tarbellica
43	Aggregatibacter aphrophilus	93	Bacillus anthracis	143	Caloramator indicus
44	Aggregatibacter pneumotropica	94	Bacillus deserti	144	Caloramator mitchellensis
45	Aggregatibacter segnis	95	Bacillus ferrariarum	145	Caloramator uzoniensis
46	Agrococcus jejuensis	96	Bacillus halmapalus	146	Caloramator viterbiensis
47	Agrococcus versicolor	97	Bacillus horneckiae	147	Calothrix brevissima
48	Agromyces hippuratus	98	Bacillus isabeliae	148	Caminibacter profundus
49	Agromyces salentinus	99	Bacillus mucilaginosus	149	Campylobacter canadensis
50	Agromyces succinolyticus	100	Bacillus olivae	150	Campylobacter concisus

151	Campylobacter curvus	201	Clostridium cadaveris	251	Demequina aurantiaca
152	Campylobacter faecalis	202	Clostridium caenicola	252	Dermacoccus profundi
153	Campylobacter fetus	203	Clostridium caliptrosporum	253	Desulfitobacterium metallireducens
154	Campylobacter gracilis	204	Clostridium cavendishii	254	Desulfobulbus elongatus
155	Campylobacter insulaenigrae	205	Clostridium fallax	255	Desulfomicrobium macestii
156	Campylobacter mucosalis	206	Clostridium frigoris	256	Desulfomicrobium norvegicum
157	Campylobacter rectus	207	Clostridium grantii	257	Desulfomicrobium orale
158	Campylobacter showae	208	Clostridium haemolyticum	258	Desulfomonile tiedjei
159	Campylobacter troglodytis	209	Clostridium histolyticum	259	Desulfonatronum thiosulfatophilum
160	Campylobacter volucris	210	Clostridium proteolyticum	260	Desulfonauticus autotrophicus
161	Candidatus Amoebophilus asiaticus	211	Clostridium proteolyticus	261	Desulfosporosinus hippei
162	Candidatus Azobacteroides pseudotrichonymphae	212	Clostridium straminisolvens	262	Desulfosporosinus lacus
163	Candidatus Blochmannia herculeanus	213	Clostridium sulfidigenes	263	Desulfotomaculum aeronauticum
164	Candidatus Contubernalis alkalaceticum	214	Clostridium taeniosporum	264	Desulfotomaculum australicum
165	Candidatus Phlomobacter fragariae	215	Clostridium tagluense	265	Desulfotomaculum indicum
166	Candidatus Phytoplasma phoenicium	216	Clostridium tepidiprofundi	266	Desulfotomaculum putei
167	Candidatus Rhabdochlamydia crassificans	217	Clostridium termitidis	267	Desulfotomaculum thermoacetoxidans
168	Candidatus Scalindua brodae	218	Clostridium thermoalcaliphilum	268	Desulfotomaculum thermobenzoicum
169	Candidatus Tammella caduceiae	219	Clostridium thermobutyricum	269	Desulfovibrio aceae
170	Capnocytophaga canimorsus	220	Clostridium thermosuccinogenes	270	Desulfovibrio brasiliensis
171	Capnocytophaga cynodegmi	221	Clostridium tunisiense	271	Desulfovibrio butyratiphilus
172	Capnocytophaga gingivalis	222	Clostridium vincentii	272	Desulfovibrio desulfuricans
173	Capnocytophaga granulosa	223	Cohnella damuensis	273	Desulfovibrio fairfieldensis
174	Capnocytophaga haemolytica	224	Cohnella fontinalis	274	Desulfovibrio ferrophilus
175	Capnocytophaga leadbetteri	225	Cohnella hongkongensis	275	Desulfovibrio idahonensis
176	Capnocytophaga ochracea	226	Cohnella laeviribosi	276	Desulfovibrio piger
177	Carboxydocella ferrireducens	227	Cohnella soli	277	Desulfovibrio psychrotolerans
178	Cardiobacterium hominis	228	Comamonas koreensis	278	Desulfovibrio simplex
179	Cardiobacterium valvarum	229	Conchiformibius kuhniae	279	Desulfurella propionica
180	Carnobacterium inhibens	230	Conchiformibius steedae	280	Desulfurispirillum alkaliphilum
181	Catenulispora yoronensis	231	Coraliomargarita akajimensis	281	Desulfurispora thermophila
182	Catonella morbi	232	Corynebacterium jeikeium	282	Desulfuromonas acetoxidans
183	Cellulomonas gelida	233	Corynebacterium kutscheri	283	Desulfuromonas svalbardensis
184	Cerasicoccus arenae	234	Corynebacterium matruchotii	284	Dethiobacter alkaliphilus
185	Cetobacterium ceti	235	Corynebacterium mustelae	285	Dialister invisus
186	Chelonobacter oris	236	Corynebacterium nuruki	286	Dialister micraerophilus
187	Chlorobaculum limnaeum	237	Corynebacterium resistens	287	Dietzia kunjamensis
188	Chromatium weissei	238	Corynebacterium ulceribovis	288	Dokdonella fugitiva
189	Chromobacterium haemolyticum	239	Corynebacterium vitaeruminis	289	Dolichospermum curvum
190	Chromobacterium piscinae	240	Crocosphaera watsonii	290	Dysgonomonas capnocytophagoides
191	Chromobacterium subtsugae	241	Cupriavidus laharis	291	Dysgonomonas hofstadii
192	Chroococcus minutus	242	Curvibacter gracilis	292	Dysgonomonas wimpennyi
193	Chryseobacterium culicis	243	Curvibacter lanceolatus	293	Ectothiorhodospira imhoffii
194	Chryseobacterium hungaricum	244	Cycloclasticus oligotrophus	294	Edaphobacter modestus
195	Chryseobacterium isbiliense	245	Dactylosporangium fulvum	295	Eggerthella sinensis
196	Chryseobacterium taichungense	246	Dactylosporangium maewongense	296	Ehrlichia ovina
197	Clostridium acidisoli	247	Dechloromonas hortensis	297	Eikenella corrodens
198	Clostridium aestuarii	248	Deferribacter autotrophicus	298	Emticicia ginsengisoli
199	Clostridium alkalicellulosi	249	Deinococcus gobiensis	299	Emticicia oligotrophica
200	Clostridium aurantibutyricum	250	Delftia lacustris	300	Enterococcus camelliae

301	Enterococcus hermanniensis	351	Haemophilus parainfluenzae	401	Leptotrichia wadei
302	Erysipelothrix inopinata	352	Haemophilus quentini	402	Lewinella lutea
303	Erysipelothrix muris	353	Halanaerobacter chitinivorans	403	Limnohabitans parvus
304	Erysipelothrix tonsillarum	354	Halanaerobium alcaliphilum	404	Listeria innocua
305	Erythrobacter aquimaris	355	Halanaerobium fermentans	405	Litoricola lipolytica
306	Euzebya tangerina	356	Halanaerobium lacurosei	406	Longilinea arvoryzae
307	Exiguobacterium soli	357	Halanaerobium praevalens	407	Luteibacter anthropi
308	Exiguobacterium taiwanense	358	Halomonas almeriensis	408	Luteococcus peritonei
309	Facklamia hominis	359	Helcococcus ovis	409	Luteolibacter algae
310	Facklamia tabacinasalis	360	Helicobacter mastomyrinus	410	Lysinibacillus parviboronicapiens
311	Ferrimicrobium acidiphilum	361	Helicobacter suncus	411	Macrococcus bovicus
312	Fibrobacter intestinalis	362	Hydrocarboniphaga daqingensis	412	Mannheimia caviae
313	Filifactor alocis	363	Hydrogenophilus denitrificans	413	Maribacter goseongensis
314	Filifactor villosus	364	Hydrogenophilus halorhabdus	414	Maricaulis indicus
315	Flammeovirga aprica	365	Hydrogenophilus hirschii	415	Marichromatium gracile
316	Flavobacterium croceum	366	Hymenobacter rigui	416	Marinitoga hydrogenitolerans
317	Flavobacterium cucumis	367	Hyphomicrobium aestuarii	417	Marinobacter arcticus
318	Flavobacterium filum	368	Janibacter limosus	418	Marinobacter salicampi
319	Flavobacterium saliperosum	369	Jeotgalicoccus nanhaiensis	419	Marinobacter squalenivorans
320	Flavobacterium swingsii	370	Johnsonella ignava	420	Marinomonas blandensis
321	Flavobacterium terrigena	371	Jonesia quinghaiensis	421	Megamonas funiformis
322	Francisella hispaniensis	372	Kingella oralis	422	Megasphaera geminatus
323	Frankia alni	373	Kitasatospora melanogena	423	Megasphaera hominis
324	Friedmanniella capsulata	374	Kosmotoga arenicorallina	424	Megasphaera micronuciformis
325	Fructobacillus pseudoficulneus	375	Kouleothrix aurantiaca	425	Megasphaera paucivorans
326	Fusobacterium canifelinum	376	Kribbella ginsengisoli	426	Megasphaera sueciensis
327	Fusobacterium gonidiaformans	377	Kushneria indalinina	427	Meiothermus granaticius
328	Fusobacterium naviforme	378	Laceyella putida	428	Mesoplasma chauliocola
329	Fusobacterium nucleatum	379	Lachnospira pectinoschiza	429	Mesoplasma entomophilum
330	Fusobacterium periodonticum	380	Lactobacillus equi	430	Metallosphaera hakonensis
331	Fusobacterium simiae	381	Lactobacillus faeni	431	Methanobrevibacter gottschalkii
332	Gallibacterium melopsittaci	382	Lactobacillus hayakitensis	432	Methylobacillus flagellatus
333	Gemella cunicula	383	Lactobacillus intermedius	433	Methylobacillus glycogenes
334	Gemella haemolysans	384	Lactobacillus secaliphilus	434	Methylonatrum kenyense
335	Gemella sanguinis	385	Lactobacillus senmaizukei	435	Methylotenera versatilis
336	Geobacillus anatolicus	386	Lactobacillus siliginis	436	Microbacterium ginsengisoli
337	Geobacillus thermoglucosidans	387	Lactococcus fujiensis	437	Microbacterium profundi
338	Geobacter pickeringii	388	Lampropedia hyalina	438	Microbacterium xinjiangensis
339	Geothrix fermentans	389	Lautropia mirabilis	439	Micrococcus endophyticus
340	Geotoga petraea	390	Legionella shakespearei	440	Micrococcus yunnanensis
341	Gillisia limnaea	391	Lentibacillus kapialis	441	Microcystis panniformis
342	Gillisia sandarakina	392	Lentibacillus salinarum	442	Micromonospora aquatica
343	Glaciecola punicea	393	Leptolyngbya laminosa	443	Micromonospora fulvoviolacea
344	Glycomyces sambucus	394	Leptospira licerasiae	444	Micromonospora rifamycinica
345	Glycomyces tenuis	395	Leptothrix discophora	445	Mitsuokella jalaludinii
346	Gramella forsetii	396	Leptotrichia buccalis	446	Mogibacterium timidum
347	Granulicatella adiacens	397	Leptotrichia goodfellowii	447	Moorella glycerini
348	Granulicatella elegans	398	Leptotrichia hofstadii	448	Moorella mulderi
349	Granulicella tundricola	399	Leptotrichia shahii	449	Moryella indoligenes
350	Haemophilus influenzae	400	Leptotrichia trevisanii	450	Muricauda lutimaris

451	Mycobacterium austroafricanum	501	Oscillatoria corallinae	551	Porphyromonas canoris
452	Mycobacterium chitae	502	Oscillospira eae	552	Porphyromonas catoniae
453	Mycobacterium diernhoferi	503	Oxalobacter vibrioformis	553	Porphyromonas circumdentaria
454	Mycobacterium gilvum	504	Paenibacillus contaminans	554	Porphyromonas endodontalis
455	Mycobacterium lepromatosis	505	Paenibacillus darangshiensis	555	Porphyromonas gingivalis
456	Mycobacterium novocastrense	506	Paenibacillus filicis	556	Porphyromonas gulae
457	Mycobacterium pinnipedii	507	Paenibacillus ginsengihumi	557	Porphyromonas macacae
458	Mycobacterium senuense	508	Paenibacillus pinihumi	558	Porphyromonas somerae
459	Mycoplasma corogypsi	509	Paenibacillus taiwanensis	559	Prevotella albensis
460	Mycoplasma edwardii	510	Parabacteroides goldsteinii	560	Prevotella amnii
461	Mycoplasma iguanae	511	Parapedobacter koreensis	561	Prevotella aurantiaca
462	Mycoplasma insons	512	Paraprevotella clara	562	Prevotella baroniae
463	Mycoplasma lipophilum	513	Paraprevotella xylaniphila	563	Prevotella bergensis
464	Mycoplasma moatsii	514	Patulibacter americanus	564	Prevotella bivia
465	Mycoplasma sualvi	515	Pectinatus cerevisiiphilus	565	Prevotella brevis
466	Mycoplasma timone	516	Pectinatus haikarae	566	Prevotella buccae
467	Mycoplasma verecundum	517	Pediococcus argentinicus	567	Prevotella buccalis
468	Myroides injenensis	518	Pedobacter agri	568	Prevotella copri
469	Myroides odoratus	519	Pedobacter daejeonensis	569	Prevotella corporis
470	Natroniella acetigena	520	Pedobacter kwangyangensis	570	Prevotella dentalis
471	Negativicoccus succinicivorans	521	Pedomicrobium australicum	571	Prevotella dentasini
472	Neisseria cinerea	522	Pelagicoccus albus	572	Prevotella denticola
473	Neisseria elongata	523	Pelagicoccus croceus	573	Prevotella enoeca
474	Neisseria flavescens	524	Pelomonas saccharophila	574	Prevotella falsenii
475	Neisseria gonorrhoeae	525	Pelotomaculum isophthalicicum	575	Prevotella histicola
476	Neisseria lactamica	526	Pelotomaculum terephthalicicum	576	Prevotella intermedia
477	Neisseria mucosa	527	Peptococcus niger	577	Prevotella loescheii
478	Neisseria polysaccharea	528	Peptoniphilus coxii	578	Prevotella maculosa
479	Neisseria subflava	529	Peptoniphilus gorbachii	579	Prevotella marshii
480	Neisseria weaveri	530	Peptoniphilus indolicus	580	Prevotella melaninogenica
481	Neorickettsia helminthoeca	531	Peptoniphilus methioninivorax	581	Prevotella micans
482	Niabella soli	532	Peptoniphilus olsenii	582	Prevotella multiformis
483	Nitrobacter hamburgensis	533	Peptostreptococcus anaerobius	583	Prevotella multisaccharivorax
484	Nitrosococcus halophilus	534	Peptostreptococcus stomatis	584	Prevotella nanceiensis
485	Nitrospina gracilis	535	Phascolarctobacterium succinatutens	585	Prevotella nigrescens
486	Nocardioides islandensis	536	Photobacterium halotolerans	586	Prevotella oralis
487	Nocardioides lentus	537	Pilimelia columellifera	587	Prevotella oris
488	Nocardiopsis terrae	538	Planifilum fimeticola	588	Prevotella oulorum
489	Nonomuraea asiatica	539	Planococcus columbae	589	Prevotella pallens
490	Nonomuraea rubra	540	Planococcus maritimus	590	Prevotella paludivivens
491	Nostoc flagelliforme	541	Planomicrobium chinense	591	Prevotella pleuritidis
492	Nostoc piscinale	542	Planomicrobium flavidum	592	Prevotella saccharolytica
493	Novosphingobium indicum	543	Planomicrobium stackebrandtii	593	Prevotella salivae
494	Novosphingobium taihuense	544	Polaribacter butkevichii	594	Prevotella shahii
495	Oceanisphaera laurenciae	545	Polynucleobacter rarus	595	Prevotella tannerae
496	Odoribacter denticanis	546	Pontibacillus chungwhensis	596	Prevotella timonensis
497	Odoribacter laneus	547	Pontibacillus halophilus	597	Prevotella veroralis
498	Oligella ureolytica	548	Pontibacter niistensis	598	Propionibacterium humerusii
499	Olsenella uli	549	Porphyromonas cangingivalis	599	Propionibacterium microaerophilum
500	Oribacterium sinus	550	Porphyromonas canis	600	Propionigenium modestum

601	Propionispora hippei	651	Selenomonas dianae	701	Streptococcus plurextorum
602	Propionivibrio pelophilus	652	Selenomonas flueggei	702	Streptococcus pneumoniae
603	Pseudomonas caricapapayae	653	Selenomonas infelix	703	Streptococcus pseudopneumoniae
604	Pseudomonas clemancea	654	Selenomonas noxia	704	Streptococcus sanguinis
605	Pseudomonas orientalis	655	Selenomonas ruminantium	705	Streptococcus sinensis
606	Pseudomonas rhodesiae	656	Serratia ureilytica	706	Streptococcus thermophilus
607	Pseudomonas teessidea	657	Shewanella pneumatophori	707	Streptococcus tigurinus
608	Pseudomonas thermotolerans	658	Shimazuella kribbensis	708	Streptococcus troglodytae
609	Pseudomonas xanthomarina	659	Slackia exigua	709	Streptococcus uberis
610	Pseudonocardia sulfidoxydans	660	Slackia faecicanis	710	Streptococcus ursoris
611	Psychrobacter glacialis	661	Slackia piriformis	711	Streptococcus vestibularis
612	Psychrobacter halophilus	662	Sneathia sanguinegens	712	Streptomyces auratus
613	Psychrobacter phenylpyruvicus	663	Snowella rosea	713	Streptomyces coriofaciens
614	Psychroflexus gondwanensis	664	Sphaerochaeta coccoides	714	Streptomyces danangensis
615	Pyramidobacter piscolens	665	Sphaerochaeta globus	715	Streptomyces goraiensis
616	Ralstonia detusculanense	666	Sphingobacterium bambusae	716	Streptomyces lazureus
617	Ralstonia insidiosa	667	Sphingobacterium shayense	717	Streptomyces nanchangensis
618	Ramlibacter tataouinensis	668	Sphingomonas molluscorum	718	Streptomyces qinlingensis
619	Rarobacter faecitabidus	669	Sphingomonas roseiflava	719	Streptomyces roseogilvus
620	Rhizobium alamii	670	Sporolactobacillus putidus	720	Streptomyces scopiformis
621	Rhodanobacter thiooxydans	671	Sporosarcina pasteurii	721	Streptosporangium purpuratum
622	Rhodobacter gluconicum	672	Sporotomaculum syntrophicum	722	Streptosporangium yunnanense
623	Rhodococcus percolatus	673	Staphylococcus intermedius	723	Succinivibrio dextrinosolvens
624	Rhodocyclus purpureus	674	Stenotrophomonas nitritireducens	724	Sulfobacillus sibiricus
625	Rhodothermus clarus	675	Streptococcus alactolyticus	725	Sulfobacillus yellowstonensis
626	Rickettsia hulinii	676	Streptococcus anginosus	726	Sulfurimonas paralvinellae
627	Rickettsia marmionii	677	Streptococcus australis	727	Sulfurospirillum deleyianum
628	Rickettsia monacensis	678	Streptococcus bovis	728	Sutterella sanguinus
629	Rikenella microfusus	679	Streptococcus castoreus	729	Symbiobacterium toebii
630	Rivularia atra	680	Streptococcus cristatus	730	Symploca atlantica
631	Robiginitalea biformata	681	Streptococcus dentapri	731	Syntrophomonas cellicola
632	Roseococcus thiosulfatophilus	682	Streptococcus dentirousetti	732	Syntrophomonas palmitatica
633	Roseomonas massiliensis	683	Streptococcus fryi	733	Syntrophomonas sapovorans
634	Roseospira mediosalina	684	Streptococcus gallinaceus	734	Tannerella forsythia
635	Rothia aeria	685	Streptococcus gordonii	735	Telmatospirillum siberiense
636	Runella limosa	686	Streptococcus halichoeri	736	Tenacibaculum japonica
637	Saccharopolyspora cebuensis	687	Streptococcus ictaluri	737	Tepidanaerobacter syntrophicus
638	Saccharopolyspora flava	688	Streptococcus infantis	738	Tepidimicrobium ferriphilum
639	Saccharothrix australiensis	689	Streptococcus intermedius	739	Tepidimonas ignava
640	Salinicoccus iranensis	690	Streptococcus lactarius	740	Tetragenococcus doogicus
641	Salinicoccus luteus	691	Streptococcus marimammalium	741	Tetragenococcus koreensis
642	Salinimicrobium terrae	692	Streptococcus milleri	742	Tetrasphaera australiensis
643	Salinivibrio budaii	693	Streptococcus mitis	743	Tetrasphaera vanveenii
644	Salisaeta longa	694	Streptococcus oligofermentans	744	Thauera aromatica
645	Scardovia wiggsiae	695	Streptococcus oralis	745	Thermacetogenium phaeum
646	Sebaldella termitidis	696	Streptococcus orisratti	746	Thermoactinomyces intermedius
647	Sedimentibacter hongkongensis	697	Streptococcus parasanguinis	747	Thermoanaerobacter acetoethylicus
648	Sedimentibacter hydroxybenzoicus	698	Streptococcus peroris	748	Thermoanaerobacter sulfurigignens
649	Segetibacter aerophilus	699	Streptococcus phocae	749	Thermoanaerobacterium islandicum
650	Selenomonas artemidis	700	Streptococcus pluranimalium	750	Thermobaculum terrenum

751	Thermodesulfatator atlanticus	801	Xanthobacter aminoxidans
752	Thermodesulfovibrio thiophilus	802	Xanthomonas oryzae
753	Thermogemmatispora foliorum	803	Xylanimicrobium pachnodae
754	Thermogemmatispora onikobensis	804	Zhihengliuella salsuginis
755	Thermosipho ferriphilus	805	Zhouia amylolytica
756	Thermovenabulum ferriorganovorum	806	Zobellia laminariae
757	Thermus rehai		
758	Thioalkalivibrio jannaschii		
759	Thiohalorhabdus denitrificans		
760	Thiomonas perometabolis		
761	Thiomonas thermosulfata		
762	Thiothrix nivea		
763	Treponema amylovorum		
764	Treponema brennaborense		
765	Treponema bryantii		
766	Treponema calligyrum		
767	Treponema denticola		
768	Treponema lecithinolyticum		
769	Treponema maltophilum		
770	Treponema medium		
771	Treponema paraluiscuniculi		
772	Treponema parvum		
773	Treponema pectinovorum		
774	Treponema porcinum		
775	Treponema putidum		
776	Treponema socranskii		
777	Treponema succinifaciens		
778	Treponema vincentii		
779	Treponema zioleckii		
780	Trichococcus flocculiformis		
781	Trichococcus pasteurii		
782	Turicibacter sanguinis		
783	Uliginosibacterium gangwonense		
784	Ureibacillus thermophilus		
785	Vagococcus salmoninarum		
786	Vagococcus teuberi		
787	Veillonella atypica		
788	Veillonella criceti		
789	Veillonella denticariosi		
790	Veillonella dispar		
791	Veillonella magna		
792	Veillonella montpellierensis		
793	Veillonella parvula		
794	Virgibacillus salexigens		
795	Viridibacillus neidei		
796	Vitreoscilla stercoraria		
797	Vogesella perlucida		
798	Weissella hanii		
799	Weissella salipiscis		
800	Weissella soli		

Characterization of Individual sample

The results of the individual samples are given in table 5, as per the total number of phyla, genera and species identified in each of the sample.

Table 5

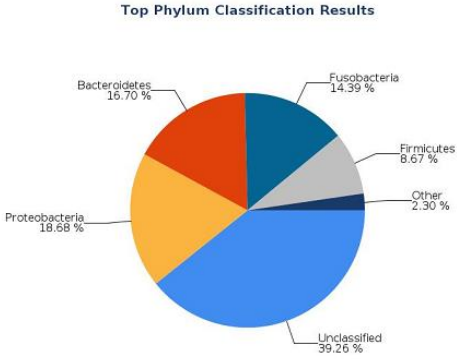
	SAMPLES	PHYLUM	GENUS	SPECIES
	1S(H)	27	487	809
	2S(H)	26	502	881
HEALTH	3S(H)	26	491	738
	4S(H)	27	502	749
	5S(D)	25	388	678
DISEASE	6S(D)	26	505	881
	7S(D)	27	478	807
	8S(D)	27	486	782

Results of most prevalent (top 8) phyla, genera and species of one health and one disease sample are represented in table 6 and table 7.

Table 6: Predominant bacterial flora at phylum, genus and species level in health sample

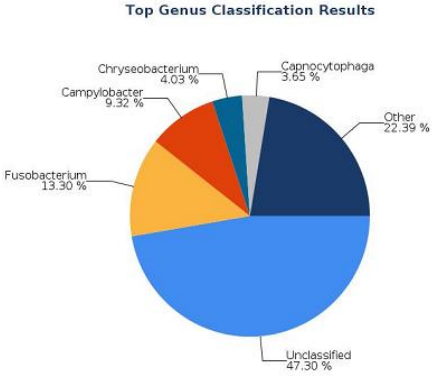
Top Phylum Classification Results

Classification	Number of Reads	% Total Reads
Unclassified at Phylum level	1,245,860	39.26 %
Proteobacteria	592,724	18.68 %
Bacteroidetes	530,005	16.70 %
Fusobacteria	456,615	14.39 %
Firmicutes	275,116	8.67 %
Actinobacteria	33,120	1.04 %
Spirochaetes	12,794	0.40 %
Thermi	10,568	0.33 %



Top Genus Classification Results

Classification	Number of Reads	% Total Reads
Unclassified at Genus level	1,501,168	47.30 %
Fusobacterium	422,021	13.30 %
Campylobacter	295,853	9.32 %
Chryseobacterium	128,009	4.03 %
Capnocytophaga	115,701	3.65 %
Cohnella	84,042	2.65 %
Leuconostoc	60,336	1.90 %
Porphyromonas	55,773	1.76 %



Top Species Classification Results

Classification	Number of Reads	% Total Reads
Unclassified at Species level	1,889,913	59.55 %
Fusobacterium naviforme	245,285	7.73 %
Campylobacter gracilis	192,988	6.08 %
Chryseobacterium taichungense	124,233	3.91 %
Fusobacterium nucleatum	55,446	1.75 %
Campylobacter showae	53,958	1.70 %
Sphingobacterium shayense	42,221	1.33 %
Zhouia amylolytica	41,545	1.31 %

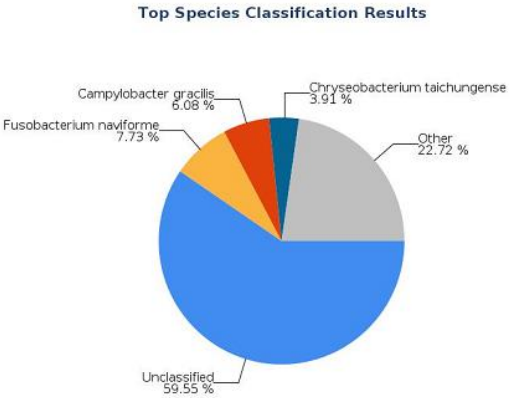
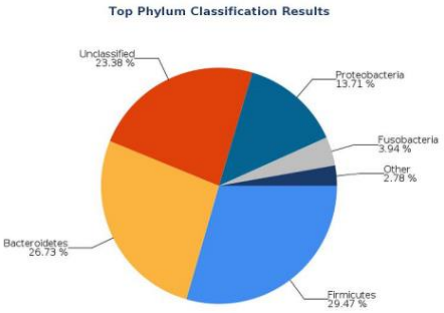


Table 7: Predominant bacterial flora at phylum, genus and species level in disease sample

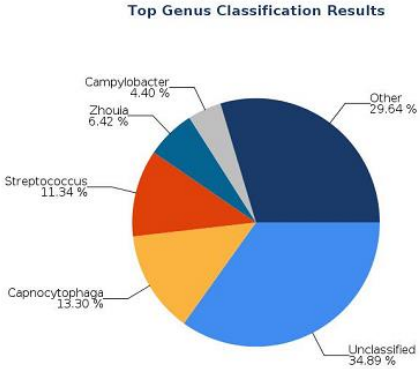
Top Phylum Classification Results

Classification	Number of Reads	% Total Reads
Firmicutes	353,676	29.47 %
Bacteroidetes	320,824	26.73 %
Unclassified at Phylum level	280,639	23.38 %
Proteobacteria	164,517	13.71 %
Fusobacteria	47,259	3.94 %
Actinobacteria	25,972	2.16 %
Thermi	5,698	0.47 %
Tenericutes	574	0.05 %



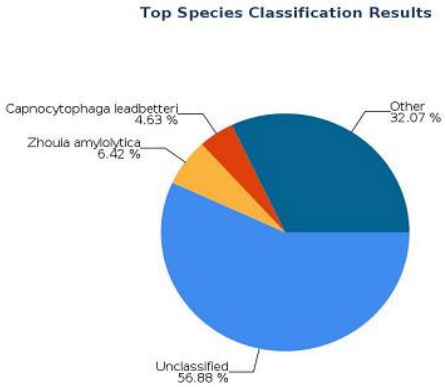
Top Genus Classification Results

Classification	Number of Reads	% Total Reads
Unclassified at Genus level	418,800	34.89 %
Capnocytophaga	159,674	13.30 %
Streptococcus	136,119	11.34 %
Zhoula	77,116	6.42 %
Campylobacter	52,832	4.40 %
Veillonella	38,031	3.17 %
Neisseria	35,764	2.98 %
Cohnella	35,446	2.95 %



Top Species Classification Results

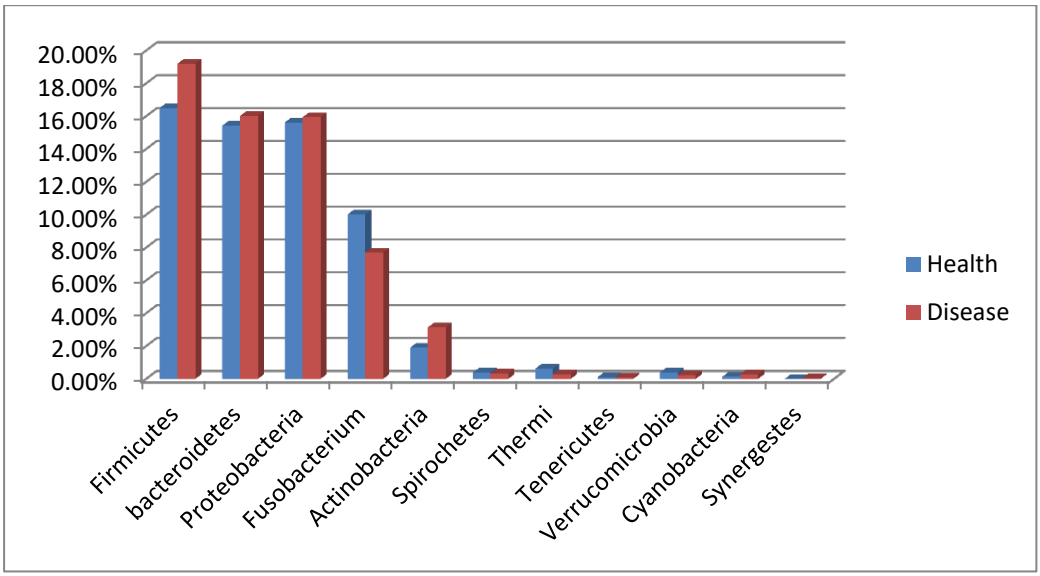
Classification	Number of Reads	% Total Reads
Unclassified at Species level	682,678	56.88 %
Zhoula amylolytica	77,115	6.42 %
Capnocytophaga leadbetteri	55,600	4.63 %
Streptococcus gordonii	29,216	2.43 %
Campylobacter curvus	19,351	1.61 %
Capnocytophaga gingivalis	17,817	1.48 %
Capnocytophaga ochracea	17,188	1.43 %
Neisseria mucosa	16,359	1.36 %



I. Comparison between health and disease

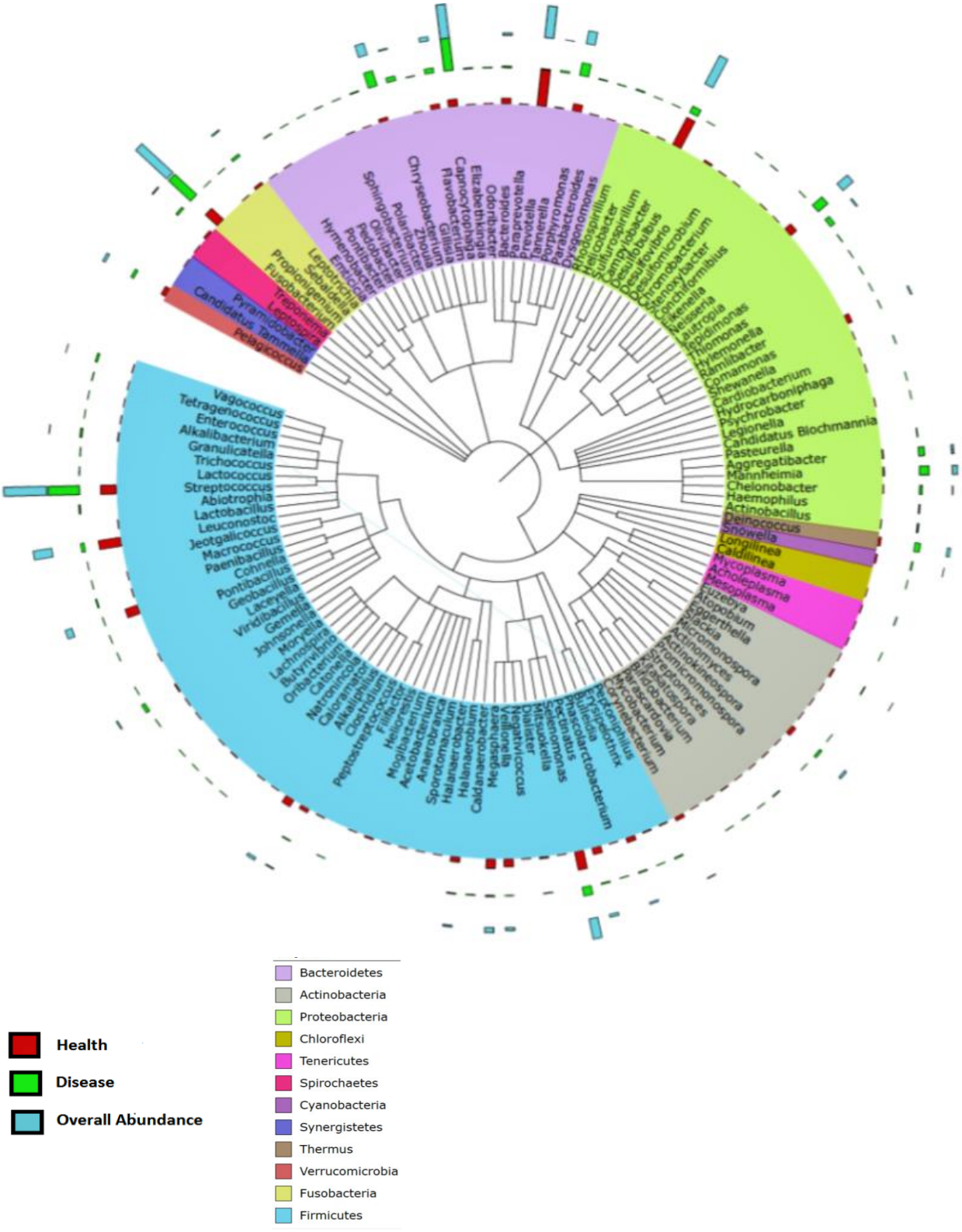
When the subgingival microbiome was compared between health and disease samples, the results at phyla level are represented in the bar graph. There was no statistically significant difference observed at phyla level.

Graph 1: Bar graph indicating the subgingival bacterial communities of healthy and periodontitis individuals at phylum level



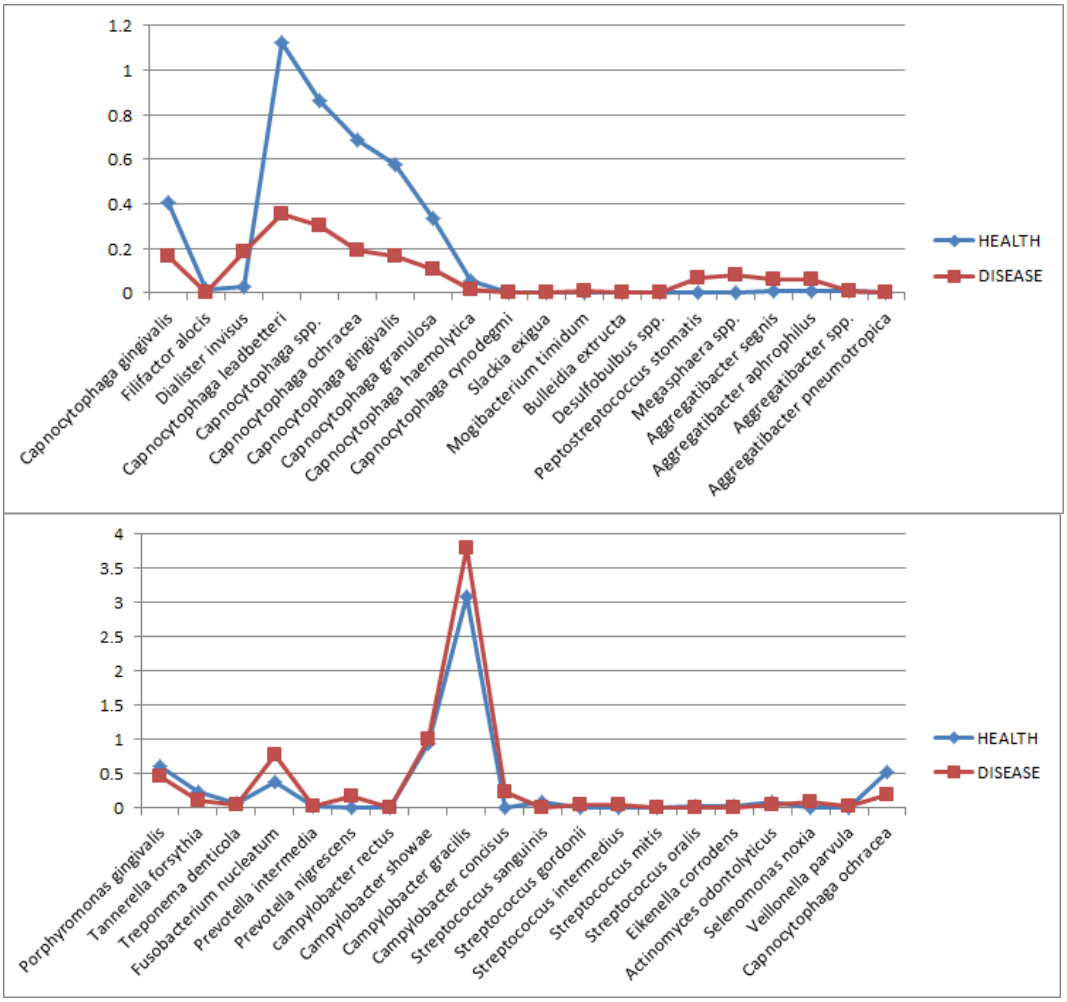
When the subgingival microbiome was compared between health and disease samples, the results at genus level is represented in circular phylogenetic tree. The tree was constructed with phyloT and displayed using iTOL (letunic and bork, 2011). The bars in the outer band (blue) represent the relative abundance of bacterial genus in the healthy (red) and the periodontal disease (green) groups.

Graph 2 : Circular maximum likelihood phylogenetic tree at the genus level.

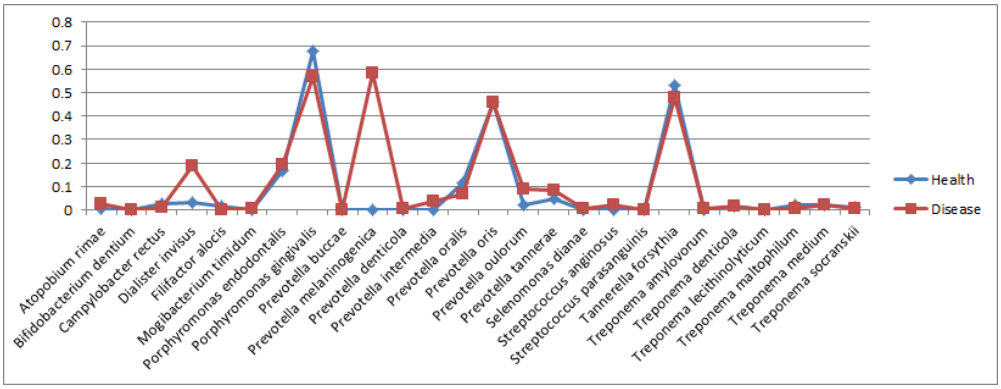


When the subgingival microbiome was compared between health and disease samples, the results of commonly associated pathogenic bacteria are represented in line graph(3). There was no significant difference in abundance of these species between health and disease.

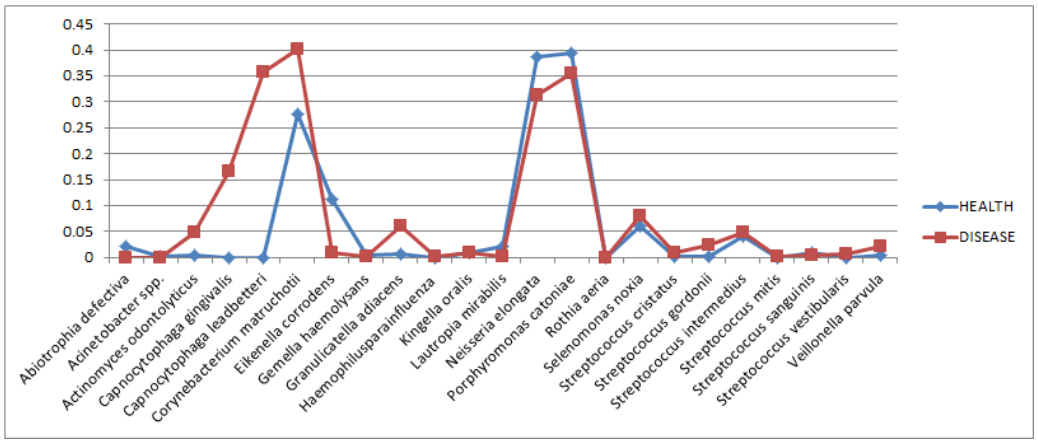
Graph 3 : Difference between health and disease at species level is shown in line graph



Graph 4: The distribution of normally health associated bacterial species in the subgingival microbiome in periodontal health and disease



Graph 5: The distribution of normally disease associated bacterial species in the subgingival microbiome in periodontal health and disease



Discussion

DISCUSSION

Periodontitis is a biofilm induced chronic inflammatory disease that leads to loss of the attachment apparatus of the periodontium (Darveau. R.P. 2010)[20]. Periodontitis has a long history of proposed microbial etiologies ranging from nonspecific plaque hypothesis to specific and keystone pathogen hypothesis. Recently, the PSD model by Hajishengallis 2012, states that polymicrobial synergy and dysbiosis in susceptible hosts causes periodontitis.[40]

The human oral microbiome refers to all the microorganisms that are found on or in the human oral cavity. The bacterial species colonizing it play an important role in oral health and disease. The oral microbiome data suggest that there are several ecological niches within the oral cavity that may have distinct microbiome of its own such as buccal mucosa, palate, tongue, tonsils etc.[23]

In the periodontal environment it has been reported that the subgingival and supragingival microbiome may be distinct from each other. The subgingival microbiome has been characterized in several populations. It is now well recognized that there is a wide variation in microbiome exists among individuals of different ethnicity and demographics.[87]

In the present study, we have used next generation sequencing, or NGS, which is the newest technology for high-throughput genomic analysis.

This methodology is in accordance with Griffen et al., Hong et al., Kumar et al., who have used NGS to characterize the subgingival microbiome.[35,45,59]

The advantages of this method include

1. The ability to identify and quantify the abundance of the entire bacterial species present in the subgingival environment. Culture based methods cannot identify species whose culture characteristics are unknown. As oral species are fastidious and need the presence of other organisms and very specific growth conditions, several species are not cultivable. It has been estimated that nearly 300 and more uncultivable species are present in subgingival plaque.[91]
2. Close ended techniques like DNA probes, RT-PCR though extremely sensitive can identify only targeted organisms against which specific primers have been designed.

Among NGS, Illumina sequencing has been used in this study for the following reasons.

1. Illumina sequencing provides more sequence per run that allows more in-depth coverage than other technologies. This in turn helps to analyse a larger sample size, inclusion of more bar-coded time points and samples, and better assessment of total diversity in microbiome.
2. Low abundance taxa can be determined with generation and sequencing of short 16S rRNA amplicons.

3. The comparatively lower cost per sequence than other competing technologies, enabling high throughput microbial ecology at the greatest coverage yet possible.[6,13]

For sample selection, periodontal examinations were performed to determine periodontal status of all subjects. Chronic periodontitis diagnosis was determined based on AAP classification parameters that includes PD ≥ 5mm in more than four sites. Healthy controls were required to have no pockets with probing depth ≤ 3mm.[113]

In this study, plaque samples were collected using sterile gracey curettes after supragingival scaling as per previous literature.

Other techniques such as paper points only allow passive translocation of plaque material and fluid into the sampling devices. This sampling method is likely to represent only the outer biofilm microorganisms, undersampling the initial colonizers present in the inner biofilm mass attached to the root surface[128]. Flavia R. Teles et al. in his study has concluded that the proportions of species remained consistent in successive curette samples, indicating that the use of curettes provided a reliable and reproducible method to obtain subgingival samples.[112] It has also been reported that endodontic paper points were potential contamination source of Enterococcus and Exiguabacterium genera.[118]

In the experimental workflow, all the reactions were carried out with water and plastic materials guaranteed as DNA-free to avoid contamination.

The results of our study for the first time characterizes the subgingival microbiome in Indian patients with periodontal disease. A total of 27 Phyla, 626 genera and 1278 species were identified with individual samples on an average exhibiting around 600 to 700 species. These results are consistent with those previously reported by Kumar et al., Griffen et al., Paster et al.[59,35,90]

The results of our study exhibit the sheer diversity of the microflora and the inter individual variation that exist from person to person. These results are also consistent with previous studies that have reported that the subgingival microbiome differs from person to person and there may not be individual bacteria that are associated with periodontal health or disease.

The results of our study indicate that the predominant phyla are Firmicutes, Bacteroidetes, Proteobacteria and Fusobacteria. Our results are consistent with those of Liu et al (2012)[66] whose whole-metagenomic data revealed a community dominated by the bacterial phyla Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria and Proteobacteria. Similar results are also reported by Kumar et al, Griffen et al.[59,35]

When the health related microbiome was analysed at phylum level, Bacteroidetes, Proteobacteria, Firmicutes and Fusobacteria predominated in

that order. These results are not in agreement with previous studies that have suggested that Firmicutes comprises the dominant phyla in health.[59]

These results are somewhat surprising because of the nature of the species belonging to these phyla. Bacteroidetes phyla consists of many of the organisms that have been associated with periodontal disease, such as, Porphyromonas, Tannerella, Capnocytophaga, Prevotella etc. However, it must be stressed that there is no significantly greater abundance of Bacteroidetes over Proteobacteria or Fermicutes. The proteobacteria phylum comprises of previously suspected periodontal pathogens such as Aggregatibacter actinomycetemcomitans and also a number of other bacterial species such as Camphylobacter, Hemophilus, Mannheimia, Desulfobulbous etc., which are which are all known to be early colonisers.[103] The Firmicutes were the third most predominant phyla in health. This is somewhat surprising, considering that the gram positive cocci that comprised the early colonisers belong to this phyla.

When the disease samples were analysed at phyla level, Firmicutes comprise the predominant phyla followed by the Bacteroidetes, Proteobacteria, and Fusobacteria. The results of the disease associated microbiome at phyla level are similar to previous studies in diseased samples.[66] Although the abundant distribution of Firmicutes in disease samples may appear somewhat surprising, the subgingival biofilm in disease

samples tends to be of greater complexity and allow for bacterial survival in even normally hostile environment.

Previous studies have reported the following advantages that bacteria obtain as a result of biofilm environment.[38,39]

1. Greater nutritional requirements are met with bacteria that are diverse at phyla level, offering metabolic products that may allow other bacteria to survive. Examples of such metabolic crosstalk have been reported with streptococci and Veillonella, Prevotella and Porphyromonas genera.
2. Transfer of Genetic material through quorum sensing and horizontal gene transfer offer a greater versatility to survive in environment that would normally be adverse. Examples that have been reported in previous studies include the acquisition of aero tolerance genes by the normally anaerobic bacteria such as Porphyromonas presumably as a result of co-colonization with aerobic bacteria such as Streptococci that allow these bacteria to reside in oxygenated environment.
3. Co-aggregation between normally diverse species at phyla level such as Streptococci gordonii and Porphyromonas gingivalis enhance the survival of these bacteria in environments that are otherwise bathed in fluid compartments like GCF and Saliva which may flush out more planktonic bacteria.

The other phyla such as spirochetes, thermi contribute only to minor proportions of subgingival microbiome. These results are in agreement with previous studies.[59,45] However, when bacteria were analysed at phyla level, TM7 was not identified in our study. Although members of this phyla are yet to be cultured or classified at species level, there have been reports that suggests the members in this phyla may play important role in disease progression.

Phylum Caldiserica is present only in health, while phylum Armatimonadetes is present only in disease. Caldiserica was initially described by Mori K as a distinct species with a new phylum, genus level classification. It was separated from candidate phyla OP5 based on its characteristics of being an anaerobic, thermophilic, filamentous bacteria with a distinct G+ C content[80]. Phylum Armatimonadetes, originally described by Tamaki as distinct from the original OP10, constituted phylogenetically diverse group of organisms that are aerobic, gram negative and exhibit oligotrophic metabolism[52]. The contribution of these bacteria to periodontal health and disease is as yet unknown.

The sheer complexity of the subgingival microbiome can be understood from the fact that the phyla predominant in health was anaerobic in nature while that in disease was aerobic which would normally not be thought as residents of the subgingival environment. These results further highlight the complex communication network that exists in subgingival biofilms.

The genus level distribution of the bacteria is represented in graph (2). In health samples, the most abundant genus comprised of Fusobacterium, Campylobacter, Capnocytophaga, Porphyromonas, Streptococci, Neisseria, Mannheimia, Chrysobacterium, Cohnella, and Leuconostoc. These results are in agreement with previous literature in as far as the distribution of Streptococci, Porphyromonas, Fusobacterium and Mannheimia are concerned.[59]

When the disease samples were analysed the most predominant bacteria at genus level were Campylobacter, Fusobacterium, Selenomonas, Corynebacterium, Capnocytophaga, Streptococci, Leuconostoc, Prevotella, Leptotrichia, Zhouia, Cohnella etc.

When comparison of genera was made at those present ≥0.5% abundance, Sphingobacterium, Tannerella, Mannheimia, Aggregatibacter, Deinococcus, Lautropia, Gemella, were present in health but not in disease. Similary, Veillonella, Actinomyces, Enterococcus, Granulicatella, Vagococcus, Corynebacterium, Treponema, Peptoniphilus, Megasphaera, Alkaliphilus, Pectinatus, Bacteroides, Halanaerobium, Pelagicoccus, Bulleidia, Clostridium, Cardiobacterium, Snowella are present only in disease when ≥ 0.5% abundance species were compared. Overall, these results are in agreement with previous literature.[35,45,59]

Subgingival microbiome at species level

At the species level, a distinct health associated microbiome (Table 3) and disease associated microbiome (Table 4) was identified.

As there are over 600 to 800 species identified in each of the 8 samples, we have discussed the most abundant ones and those commonly associated with periodontal health and disease in greater detail.

When the bacteria were analysed at the species level the 10 most abundant species were Fusobacterium naviforme, Campylobacter gracilis, Chryseobacterium taichungense, Zhouia amylolytica, Capnocytophaga leadbetteri, Corynebacterium matruchotii, Streptococcus gordonni, Fusobacterium nucleatum, Leptotrichia trevisanii, Selenomonas infelix, Campylobacter showae, Mannheimia caviae, Alkaliphilus crotonatoxidans, Pectinatus cerevisiiphilus. As is obvious, several of these species are not that have been previously thought to be periopathogenic species or health associated ones.

The red complex bacteria, Porphyromonas gingivalis, Tannerella forsythia were detected in all the samples but there was no significant different in its presence in health and disease. These results are not in agreement with Socransky and Haffajee[103], Ximenez-Fyvie et al.[125] who have described red complex bacteria as climax colonizers and thought to be most associated with periodontal disease. Treponema denticola was not identified in one health and

one disease sample but there was no significant difference between its presence in other health and disease samples. These results are however in agreement with Kumar et al., Griffen et al., etc.[59,35] who have shown that there are no significant difference in the presence of red complex bacteria in health and disease when the microbiome as a whole was studied. These authors have concluded that it is the targeted primer approach and a consequence of the pocket environment that led to the increased presence of red complex bacteria described in previous periodontal literatures.[59]

The red complex bacteria are gram negative, anerobic, proteolytic bacteria that reside in the favourable environment of a periodontal pocket. They have been closely associated with deep periodontal pockets and bleeding on probing in previous literature. The samples that have been chosen in our study were also taken from sites with periodontal pocket of greater than 5 mm which exhibited bleeding on probing. Therefore, our results are unlikely to have been influenced by sampling techniques or further microbial detection methods.

If our results are to be interpreted, the red complex bacteria seem to play no/limited role in the traditional pathogens etiopathogenesis of periodontal disease. However, it may be argued that red complex bacteria may act as keystone pathogens that even in low abundance play an important role in organizing the subgingival biofilm and subverting the immune responses.

Both events are thought to be central to the pathogenesis of periodontal disease.

The bridging organism Fusobacterium nucleatum has been described as important for co-aggregation between the early and the late colonizers, both to Streptococcus and Porphyromonas gingivalis, thereby helping the organization of the subgingival biofilm. It has been suggested this organism may be used as marker for transition from gingivitis to periodontitis and for further disease progression[110]. However the results of our study show no significant difference in its distribution between health and disease suggesting a limited role in disease pathogenesis. Our results are in agreement with previous subgingival microbiome reports, which do not consider Fusobacterium nucleatum to be a major periodontopathogen.[35]

Aggregatibacter aphrophilus, Aggregatibacter segnis, Aggregatibacter neumotropica were identified in both health and disease samples with no significant difference. Aggregatibacter actinomycetemcomitans has been described as a putative periodontal pathogen based on its leukotoxin producing ability, tissue invasiveness and high collagenolytic activity. Surprisingly however none of our samples showed the presence of Aggregatibacter actinomycetemcomitans. Previous periodontal literature has associated Aggregatibacter actinomycetemcomitans with localized juvenile periodontitis, later classified as aggressive periodontitis[78]. As the samples in our study were chosen from patients diagnosed with chronic periodontitis, it is perhaps not

surprising that Aggregatibacter actinomycetemcomitans was not identified in our samples as a dominant pathogen. Even so, our results are not in agreement with previous literature that have demonstrated presence of this bacteria in chronic periodontitis.[104] The reasons for this discrepancy are not immediately apparent.

The Streptococci mainly the Streptococcus sanguinis, Streptococcus gordonii, Streptococcus mitis, Streptococcus intermedius, and Streptococcus oralis are also part of early colonizers that have been described by Sockransky, Haffajee.[103] These bacteria are gram positive, aerobic, carbohydrate utilizing bacteria which are able to colonize on the acquired pellicle on tooth surfaces, initiating plaque formation. These bacteria utilize the available resources, create a bacterial succession through an ecological shift as described by Marsh and contribute to formation of late colonizers.[72] The results of our study suggest that there is no difference in the prevalence of streptococci between health and disease. These results are in agreement with previous literature in relation to subgingival microbiome.[59]

Newer periodontopathogenic bacteria such as Filifactor alocis and Dialister invisus have been described as being important for progressive periodontitis.[59] The results of our study however show that there is no difference in the distribution of either Filifactor alocis or Dialister invisus, suggesting that these organisms may not play major role in etiopathogenesis.[35,38]

Eikenella corrodens, Capnocytophaga species, Selenomonas noxia, Megasphaera species, Campylobacter rectus, Prevotella intermedia, all bacteria that have been described as periodontopathogens, showed no difference in abundance in health and disease samples of our study. Similar results have been described by other authors.[35,38]

Novel subgingival species were identified to be present abundantly in all the samples that were examined in our study including Zhouia amylolytica, Chryseobacterium taichungense, Leptotrichia trevisanii, Alkaliphilus crotonatoxidans, and Pectinatus cerevisiiphilus.

Zhouia amylolytica, first reported by Liu ZP, is an aerobic bacilli that require sodium chloride for its growth. This bacteria utilizes carbohydrates and is incapable of reducing the nitrates. The implication of the presence of the organism is not immediately obvious. It may be the dietary pattern of our study population which is largely rice consuming and the salt content in water that may have allowed the species to colonize the gingival environment study population belongs to coastal area.

Chrysobacterium taichungense and Leptotrichia trevisanii are gram negative anaerobes present abundantly in all the samples.

Chrysobacterium taichungense, first isolated from contaminated soil is positive for gelatinase activity and grows well over a broad range of pH values

(6.0–9.0) but grows better in neutral or weakly alkaline conditions (pH 7.0–8.0).[28]

Leptotrichia trevisanii has been reported as normal oral flora and also resides in GIT and female genital tract. It is highly sacchrolytic and produce lactate as sole end product of glucose fermentation. It has been reported as a possible pathogen in immunocompromised patients and has been reported to cause bacteraemia in such patients. Leptotrichia sp., has been frequently studied with regard to the cervicovaginal microflora.[65]

Alkaliphilus crotonotoxidans is present predominantly in disease, but either absent or less abundant in health. It is a gram positive, anaerobe belonging to phylum Firmicutes. They utilize only proteinaceous substance such as yeast extract peptone, tryptone, as sole source of energy. It grows well in the optimum pH of 7.5.[12]

Pectinatus cerevisiphilus is also predominantly present in disease, but not in health. This species is a gram negative anaerobe, considered a common beer spoilage bacteria. They produce propionate as a major fermentation product. They are also reported to be isolated from drainage systems and water pipe systems. It grows well in the optimum pH of 6-6.2.[115]

In our results support the hypothesis that the subgingival biofilm as a whole and dysbiosis may contribute more to the pathogenesis of periodontal disease rather than individual bacteria. There was a distinct bacterial species in

disease associated microbiome when compared to health (Table 3,4). The traditional periodontal pathogens (red complex bacteria and newer Filifactor, Dialister) seem to have a limited role in disease pathogenesis. Novel bacteria Alkaliphilus crotonoxidans and Pectinatus cerevisiphilus seem to be closely associated with periodontitis but further studies need to be done to ascertain their etiopathogenic role. The results indicate that our dietary and lifestyle habits could have contributed to a microbial profile that has not been reported in previous literature. The greater carbohydrate content in our diet could have allowed the presence of normal sacchrolytic bacteria and a neutral to mildly alkaline pH environment could have favoured the growth novel subgingival species such Zhouia amylolytica, Chryseobacterium taichungense, Leptotrichia trevisanii, Alkaliphilus crotonatoxidans, and Pectinatus cerevisiiphilus.

The limitations of this study include the small sample size and the lack of exact quantification of the bacterial species which cannot be done even using the NGS technology. In any case these results reaffirm that targeted antimicrobial approach against individual or group of bacteria may not be ideal for management of periodontal disease.

Summary and Conclusion

SUMMARY AND CONCLUSION

This study was carried out to characterize the subgingival microbiome in periodontitis and to compare it with health. 8 subgingival samples including 4 healthy and 4 chronic periodontitis samples were collected and microbiome characterization was done with NGS technology using Illumina sequencing.

A total number of phyla identified were 27, genera 626 and species 1278 from all the samples that have been collected, with individual samples showing between 600 and 800 bacterial species.

When subgingival microbiome was characterized, most of the bacterial species belonged to previously described phyla and genera.

On comparison, distinct health and disease associated microbiome was identified. Traditional periodonto pathogenic bacteria such as Porphyromonas gingivalis, Tannerella Forsythia, Treponema denticola, Capnocytophaga species, Selenomonas noxia, Prevotella intermedia and newer periodonto pathogens such as Filifactor alocis, Dialister invisus showed no significant difference on abundance and behaviour in health and disease.

Novel subgingival bacterial species were identified in both health and periodontitis samples. Bacterial species such as Alkaliphilus crotonatoxidans, Pectinatus cerevisiiphilus were more abundant in disease and absent or less abundant in health. Further studies need to be done to identify the role of these bacteria in periodontal health and disease.

Bibliography

BIBLIOGRAPHY

1. **Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE.** Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005: 43: 5721–5732.
2. **Adessi C, Matton G, Ayala G, Turcatti G, Mermod JJ, Mayer P, Kawashima E.** Solid phase DNA amplification: characterization of primer attachment and amplification mechanisms. Nucleic Acids Res 2000: 28: e87.
3. **Aoi Y, Kinoshita T, Hata T, Ohta H, Obokata H, Tsuneda S.** Hollow-fiber membrane chamber as a device for in situ environmental cultivation. Appl Environ Microbiol (2009);75: 3826-3833.
4. **Avila, M., Ojcius, D. M. & Yilmaz, O.** The oral microbiota: living with a permanent guest. DNA and Cellular Biology,(2009);28, 405-411.
5. **Badger JH, Ng PC, Venter JC.** The human genome, microbiomes, and disease. In: Nelson KE, ed. Metagenomics of the human body. Springer Science Business Media: New York, (2011):vol 17, pp. 1–14.
6. **Bartram A.** "Generation of multimillion 16S rRNA gene libraries from complex microbial communities by assembling pairedend Illumina reads." Appl Environ Microbiol (2011);77(15):5569.
7. **Becker, M.R**. Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol, 2002. 40(3): p. 1001-9.

8. **Bentley DR**. Whole-genome resequencing. Curr Opin Genet Dev. 2006;16:545-52.
9. **Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, et al.(2008)**: Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53-59.
10. **Black,G.V.(1884).** The Formation of Poisons by Micro-Organisms. A Biological Study of the Germ Theory of Disease. Philadelphia, PA : P.Blakiston,son&co. doi: 10.5962/bhl.title.21164
11. **Bollmann A, Lewis K, Epstein SS (2007):** Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates. Appl Environ Microbiol; 73:6386-90.
12. **Cao X, Liu X, Dong X,** Alkaliphilus crotonatoxidans sp. nov., a strictly anaerobic, crotonate-dismutating bacterium isolated from a methanogenic environment, Int J Syst Evol Microbiol. 2003 Jul;53(Pt 4):971-5.
13. **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD(2010)** QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7: 335-336.
14. **Chen, H. & Jiang, W. (2014).** Application of high-throughput sequencing in understanding human oral microbiome related with health and disease. Frontiers in Microbiology, 5, 508.

15. **Chiang, PW, Song WJ, Wu KY, korenberg JR, Fogel EJ, Van Keuren ML, Lashkari D, and Kurnit DM (1996):** Use of fluorescent-PCR reaction to detect genomic sequence copy number and transcriptional abundance. Genome Res. 6:1013-1026
16. **Chien A, David BE and John MT (1976):** Deoxyribonucleic acid polymerase from the Extreme Thermophile Termus aquaticus. J Bacteriology. 127: 1550- 1557.
17. **Chi-Ying Tsai , Chuan Yi Tang , Te-Sheng Tan , Kuan-Hsueh Chen , Ki-Hok Liao, Ming-Li Liou,** Subgingival microbiota in individuals with severe chronic periodontitis, Journal of Microbiology, Immunology and Infection (2016) xx, 1-9
18. **Clarridge JE (2004):** Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev. 17: 840-62.
19. **Colyer JF.** Progressive destruction of the teeth. In: Colyer JE ed. Dental surgery and pathology. London: Longmans Green and Co., 1919: 560-561, 594-596.
20. **Darveau, R.P.(2010).** Periodontitis: a polymicrobial disruption of host homeostasis. Nat. Rev. Microbiol. 8, 481–490.doi:10.1038/nrmicro2337
21. **De Lillo, A.** Culture-independent identification of periodontitis-associated Porphyromonas and Tannerella populations by targeted molecular analysis. J Clin Microbiol, 2004. 42(12): p. 5523-7.

22. **Dethlefsen L, McFall-Ngai M, Relman DA (2007).** An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 449: 811–818.

23. **Dewhirst FE, Chen T, Izard J (2010).** The human oral microbiome. J Bacteriol 192: 5002–5017.

24. **Dohm JC, Lottaz C, Borodina T, Himmelbauer H**. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. Nucleic Acids Res 2008: 36: e105.

25. **Dzink, J.L., Tanner,A.C., Haffajee, A.D. and Socransky, S.S. (1985)** Gram negative species associated with active destructive periodontal lesions. J. Clin. Periodontol., 12, 648–659.

26. **Dzink, J.L., Socransky, S.S. and Haffajee, A.D. (1988)** The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. J. Clin. Periodontol., 15, 316–323.

27. **Feres, M.** Change in subgingival microbial profiles in adult periodontitis subjects receiving either systemically-administered amoxicillin or metronidazole. J Clin Periodontol, 2001. 28(7): p. 597-609.

28. **Fo-Ting Shen, Peter Kampfer, Chiu-Chung Young, Wei-An Lai and A. B. Arun** Chryseobacterium taichungense sp. nov.,isolated from contaminated soil, international Journal of Systematic and Evolutionary Microbiology (2005), 55, 1301–1304

29. **Frank, D.N.** Culture-independent molecular analysis of microbial constituents of the healthy human outer ear. J Clin Microbiol, 2003. 41(1): p. 295-303.
30. **Gavrish E, Bollmann A, Epstein S, Lewis K (2008):** A trap for in situ cultivation of filamentous actinobacteria. J Microbiol Methods; 72: 257-62.
31. **Ghannoum, M. A., Jurevic, R. J., Mukherjee, P. K., Cui, F., Sikaroodi, M., Naqvi, A. & Gillevet, P. M. (2010).** Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathogens, 6, e1000713.
32. **Gibson, UE, Heid CAand Williams PM (1996):** A novel method for real time quantitative PCR. Genome Res.6:986-994.
33. **Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE.** Metagenomic analysis of the human distal gut microbiome. Science 2006: 312: 1355–1359
34. **Gmur, R. and B. Guggenheim,** Monoclonal antibodies for the detection of 'periodontopathic' bacteria. Arch Oral Biol, 1990. 35 Suppl: p. 145S-151S.
35. **Griffen, A.L.,** Prevalence of Porphyromonas gingivalis and periodontal health status. J Clin Microbiol, 1998. 36(11): p. 3239-3242.

36. **Haffajee. A. D. & Socransky, S, S. (1994)** Microbial etiological agents of destructive periodontal diseases. In: Socransky. S,S & Haffajee. A, D. ed. Microbiology and Immunology of periodontal diseases, Periodontology 2000, 5 ,78-111,

37. **Haffajee, A.D.,** Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. J Clin Periodontol, 1998. 25(5): p. 346-53. 5.

38. **Haffajee AD, Bogren A, Hasturk H, Feres M, Lopez NJ, and Socransky SS**: Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol 2004; 31(11): 996–1002. PMID: 15491316

39. **Hajishengallis,G.,Darveau,R.P.,andCurtis,M.A.(2012).**Thekeystone -pathogen hypothesis. Nat.Rev.Microbiol. 10, 717–725.doi:10.1038/nrmicro2873

40. **Hajishengallis,G.,andLamont,R.J.(2012).**Beyondtheredcomplexandi nto more complexity : the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol.OralMicrobiol. 27, 409–419.doi:10.1111/j.2041- 1014.2012.00663.x

41. **Harismendy O, Ng PC, Strausberg RL, Wang X, Stockwell TB, Beeson KY, Schork NJ, Murray SS, Topol EJ, Levy S, Frazer KA**. Evaluation of next generation sequencing platforms for population targeted sequencing studies. Genome Biol 2009: 10: R32.

42. **Heid CA, Stevens J, Livak KJ, Williams PM (1996):** Real time quantitative PCR. Genome Res. 6:986-994.

43. **Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J (2007):** qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol;8:R19.

44. **Henry A. Erlich (1989):** Polymerase Chain Reaction. J Clin. Immunology 9:(6) 235-239

45. **Hong BY, Furtado Araujo MV, Strausbaugh LD, Terzi E, Ioannidou E, Diaz PI.** Microbiome profi les in periodontitis in relation to host and disease characteristics. PLoS One 2015; 10(5): e0127077.

46. **Huang S, Li R, Zeng X, He T, Zhao H, Chang A, Bo C, Chen J, Yang F, Knight R, Liu J, Davis C, Xu J.** Predictive modeling of gingivitis severity and susceptibility via oral microbiota. ISME J 2014; 8(9): 1768-80.

47. **Huber JA, Morrison HG, Huse SM, Neal PR, Sogin ML, Mark Welch DB.** Effect of PCR amplicon size on assessments of clone library microbial diversity and community structure. Environ Microbiol. 2009; 11:1292–1302. [PubMed: 19220394]

48. **Jennifer J. Barb, Andrew J. Oler, Hyung-Suk Kim, Natalia Chalmers, Gwenyth R. Wallen, Ann Cashion, Peter J. Munson1,**

Nancy J. Ames PLOS ONE | DOI:10.1371/journal.pone.0148047 February 1, 2016

49. **Kaeberlein T, Lewis K, Epstein SS (2002):** Isolating 'uncultivable' microorganisms in pure culture in a simulated natural environment. Science; 296: 1127-9.
50. **Kazor CE, Mitchell PM, Lee AM et al (2003):** Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. J Clin Microbiol 41: 558-563.
51. **Keijser BJ, Zaura E, Huse SE, van der Vossen JM, Schuren FH, Montijn RC, ten Cate JM and Crielaard W (2008):** Pyrosequencing analysis of the Oral Microflora of healthy adults. Journal of Dental Research. 87: 1016-1020.
52. **Kevin C. Y. Lee , Peter F. Dunfield, Matthew B. Stott, October 2014,** The Phylum Armatimonadetes, The Prokaryotes, pp 447-458
53. **Keyes, P. H. & Jordan, H. V. (1964).** Periodontal lesions in the Syrian hamster.Ⅲ. Findings related to an infectious and transmissible component. Archives of Oral Biology, 9, 377-400.
54. **Kirst ME, Li EC, Alfant B, Chi YY, Walker C, Magnusson I, Wang GP.** Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. Appl Environ Microbiol 2015; 81(2): 783-93.

55. **Kistler JO, Booth V, Bradshaw DJ, Wade WG.** Bacterial community development in experimental gingivitis. PLoS One 2013; 8(8): e71227

56. **Kolenbrander, P. E. (2000).** Oral microbial communities: biofilms, interactions, and genetic systems. Annual Reviews in Microbiology, 54, 413-437.

57. **Korbel JO, Urban AE, Affourtit JP, Godwin B, Grubert F, Simons JF, (2007):** Paired-end mapping reveals extensive structural variation in the human genome. Science;318:420-6.

58. **Kroes I, Lepp PW, Relman DA.** Bacterial diversity within the human subgingival crevice. Proc Natl Acad Sci U S A 1999; 96(25): 14547-52.

59. **Kumar PS, Griffen AL, Moeschberger ML, Leys EJ.** Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. J Clin Microbiol. 2005 Aug; 43(8):3944–3955. [PubMed: 16081935]

60. **Kumar PS, Leys EJ, Bryk JM, Martinez FJ, Mosechberger ML, and Griffen AL (2006):** Changes in periodontal health status are associated with bacterial community shifts as assessed by quantitative 16S cloning sequencing. J. Clin.Microbiol.44, 3665-3673.

61. **Lamont RJ1, Hajishengallis** Polymicrobial synergy and dysbiosis in inflammatory disease., Trends Mol Med. 2015 Mar;21(3):172-83. doi: 10.1016/j.molmed.2014.11.004. Epub 2014 Nov 20.

62. **Lederberg J, McCray A.** Ome sweet 'omics: A genealogical treasury of words. The Scientist. 2001;15:8.

63. **Letunic I, Bork P. (2011).** Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. Nucleic Acids Res 39: W475–W478.

64. **Leys, E.J.,** Association of Bacteroides forsythus and a novel Bacteroides phylotype with periodontitis. J Clin Microbiol, 2002. 40(3): p. 821-5.

65. **Ling Z, Liu X, Chen X,.** Diversity of cervicovaginal microbiota associated with female lower genital tract infections. Microb Ecol 2011; 61(3): 704-14.

66. **Liu WT, Marsh TL, Cheng H, Forney LJ.** Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 1997: 63: 4516–4522.

67. **Loe,H.,Theilade,E.,andJensen,S.B.(1965).**Experimentalgingivitisinman. J. Periodontol. 36, 177–187.doi:10.1902/jop.1965.36.3.177 .

68. **Loesche WJ. Syed SA. Stoll J.** Trypsin-like activity in subgingival plaque. A diagnostic marker for spirochetes and periodontal disease?. Journal of Periodontology. 58(4):266-73, 1987 bacteria: can culture be considered the primary reference standard? J Clin Microbiol, 1992. 30(2): p. 418-26.

69. **MacLean D, Jones JD, Studholme DJ.** Application of next-generation sequencing technologies to microbial genetics. Nat Rev Microbiol 2009: 7: 287–296.

70. **Mardis ER (2008):** Next-Generation DNA Sequencing Methods Annu. Rev. Genomics Hum. Genet. 9:387-402

71. **Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, et al. (2005):** Genome sequencing in microfabricated high-dentisty picolitre reactors. Nature. 437:376-80

72. **Marsh, P.D.(1994).** Microbialecology of dental plaquean ditssignificancein health anddisease. Adv. Dent.Res. 8, 263–271.

73. **Miller, W.D.(1890).** The Micro-Organisms of the Human Mouth. Philadelphia, PA: The S.S.WhiteDentalMFG.CO.

74. **Mitchell, D. F. & Johnson, M. (1956).** The nature of the gingival plaque in the hamster-production, prevention, and removal. Journal of Dental Research, 35, 651-655.

75. **Metzker ML.** Sequencing technologies – the next generation. Nat Rev Genet 2010: 11: 31–46.

76. **Moore. W. E, C & Moore. L. V H, (1994)** The bacteria of periodontal diseases. Periodontology 2000 5. 66 77.

77. **Moore, W.E., Holdeman, L.V., Cato, E.P (1983)** Bacteriology of moderate (chronic) periodontitis in mature adult humans. Infect. Immun., 42, 510–515.

78. **Moore, W.E., Holdeman, L.V., Smibert, R.M. (1982)** Bacteriology of severe periodontitis in young adult humans. Infect. Immun., 38, 1137–1148.

79. **Morgan, Curtis Huttenhower, Chapter 12:** Human Microbiome Analysis, PLOS Computational Biology, December 2012 | Volume 8 | Issue 12 | e1002808

80. **Mori K, Kaoru Yamaguchi, Yayoi Sakiyama, Tetsuro Urabe and Ken-ichiro Suzuki,** Caldisericum exile gen. nov., sp. nov., an anaerobic, thermophilic, filamentous bacterium of a novel bacterial phylum, Caldiserica phyl. nov., originally called the candidate phylum OP5, and description of Caldisericaceae fam. nov., Caldisericales ord. nov. and Caldisericia classis nov., International Journal of Systematic and Evolutionary Microbiology (2009), 59, 2894–2898

81. **Mullis KB, Faloona F (1987):** Specific synthesis of DNA in vitro via a polymerase catalysed chain reaction. Meth Enzymol. 155:335-350

82. **Newman, M. A., S. S. Socransky, E. D. Savitt, D. A. Propas, and A. Crawford. 1976**. Studies of the microbiology of periodontosis. J. Periodontol. 47:373-379.

83. **Nossa CW, Oberdorf WE, Yang L, Aas JA, Paster BJ, (2010):** Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J Gastroenterol 16: 4135-4144.

84. **Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, Miura K, Kozai K.** Longitudinal study of dental caries incidence associated with

Streptococcus mutans and Streptococcus sobrinus in pre-schoolchildren. J Med Microbiol. 2005; 54:661–665. [PubMed: 15947431]

85. **Pace, N.R.,** A molecular view of microbial diversity and the biosphere. Science, 1997. 276(5313): p. 734-40.
86. **Page, R.C., Lantz, M.S., Darveau, R., Jeffcoat, M., Mancl, L., Houston, L (2007).** Immunization of Macaca fascicularis against experimental periodontitis using a vaccine containing cysteine proteases purified from Porphyromonas gingivalis. OralMicrobiol.Immunol. 22, 162–168.doi: 10.1111/j.1399-302X.2007.00337.x
87. **Park OJ, Yi H, Jeon JH, Kang SS, Koo KT, Kum KY, Chun J, Yun CH, Han SH.** Pyrosequencing Analysis of Subgingival Microbiota in Distinct Periodontal Conditions. J Dent Res 2015; 94(7): 921-7.
88. **Paster BJ, Bartoszyk IM, Dewhirst FE**. Identification of oral streptococci using PCR-based, reverse-capture, checkerboard hybridization. Methods Cell Sci. 1998; 20:223–231.
89. **Paster, B.J.,** Prevalent bacterial species and novel phylotypes in advanced noma lesions. J Clin Microbiol, 2002. 40(6): p. 2187-91.
90. **Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A**, Dewhirst FE. Bacterial diversity in human subgingival plaque. J Bacteriol 2001; 183(12): 3770-83.

91. **Peterson, J., Garges, S. & Giovanni, M. (2009).** The NIH Human Microbiome Project. Genome Research, 19, 2317-2323.

92. **Preza D, Olsen I, Aas JA, Willumsen T, Grinde B, Paster BJ.** Bacterial profiles of root caries in elderly patients. J Clin Microbiol 2008: 46: 2015–2021.

93. **Rajendhran J, Gunasekaran P (2010).** Human microbiomics. Indian J Microbiol 50: 109–112.

94. **Riggio MP, Lennon A, Rolph HJ, Hodge PJ, Donaldson A, Maxwell AJ, (2008):** Molecular identification of bacteria on the tongue dorsum of subjects with and without halitosis. Oral Dis 14:251-258.

95. **Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M, Leamon JH, Johnson K, Milgrew MJ, Edwards M,:** An integrated semiconductor device enabling non-optical genome sequencing. Nature 2011, 475(7356):348-352.

96. **Saiki RK, Gelfand DH, Stoffel S, Scharf s, Higuchi RH, Horn GT, Mullis KB, Erlich HA (1988):** Primer-directed enzymatic amplification of DNA with a thermostable DNA Polymerase. Science 239:487-491.

97. **Samaranayake, L. P. (2012).** Essential microbiology for dentistry. 4th edition. Edinburgh: Churchill Livingstone.

98. **Sanz M, Lau L, Herrera D, Morillo JM, Silva A.** Methods of detection of Actinobacillus actinomycetemcomitans, Porphyromonas

gingivalis and Tannerella forsythensis in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. J Clin Periodontol. 2004; 31:1034–1047. [PubMed: 15560803]

99. **Siqueira J. F. & Rocas I. N. (2010).** The oral microbiota: general overview, taxonomy, and nucleic acid techniques. Methods Molecular Biology, 666, 55–69.
100. **Sizova MV, Hohmann T, Hazen A, Paster BJ, Halem SR, Murphy CM, (2012):** New approaches for isolation of previously uncultivated oral bacteria. Appl Environ Microbiol 78:194-203
101. **Slots, J. 1976.** The predominant cultivable organisms in juvenile periodontitis. Scand. J. Dent. Res. 84:1-10.
102. **Socransky, S.S. and Haffajee, A.D. (1994**) Evidence of bacterial etiology: a historical perspective. Periodontology 2000, 5, 7–25.
103. **Socransky,S.S.,Haffajee,A.D.,Cugini,M.A.,Smith,C.,andKent,R.L. Jr.(1998).** Microbial complexes in subgingival plaque. J. Clin. Periodontol. 25, 134–144. doi: 10.1111/j.1600-051X.1998.tb02419.x
104. **Socransky, S.S., Tanner, A.C.R., Haffajee, A.D., Hillman, J.D., and Goodson, J.M. (1982).** Present Status of Studies on the Microbial Etiology of Periodontal Diseases. Washington, DC: American Society for Microbiology.
105. **Socransky, S.S., C. Smith, and A.D.** Haffajee, Subgingival microbial profiles in refractory periodontal disease. J Clin Periodontol, 2002. 29(3): p. 260-8.

106. **Sonnenburg JL, Fischbach MA (2011).** Community health care: therapeutic opportunities in the human microbiome. Sci Transl Med 3: 1–5.

107. **Spratt DA (2004):** Significance of bacterial identification by molecular biology methods. Endodontic Topics. 9, 5-14.

108. **Tal. M, (1980)** Periodontal disease and oral hygiene, De.scribed by Antonic van Leeuwenhoek, Journal of Periodontologv, 51, 668-669.

109. **Tanner, A.C., Haffer, C., Bratthall, G.T. (1979)** A study of the bacteria associated with advancing periodontitis in man. J. Clin. Periodontol., 6, 278–307.

110. **Tanner, A., Maiden, M.F., Macuch, P.J. (1998)** Microbiota of health, gingivitis, and initial periodontitis. J. Clin. Periodontol., 25, 85–98.

111. **Tanner, A.,** The impact of 16S ribosomal RNA-based phylogeny on the taxonomy of oral bacteria. Periodontol 2000, 1994. 5: p. 26-51.

112. **Teles R, Sakellari D, Teles F, Konstantinidis A, Kent R, Socransky S (2010)**. Relationships among gingival crevicular fluid biomarkers, clinical parameters of periodontal disease, and the subgingival microbiota. J Periodontol 81: 89–98.

113. **The American Academy of Periodontology.** Guidelines for Periodontal Therapy (Position Paper). J Periodontol 1998;69:396-399.

114. **Theilade, E.(1986)**.Thenon-specific theory in microbial etiology of inflammatory periodontal diseases. J. Clin. Periodontol. 13, 905–911.doi:10.1111/j.1600- 051X.1986.tb01425.x

115. **Tholozan JL, Membré JM, Grivet JP.,** Physiology and development of Pectinatus cerevisiiphilus and Pectinatus frisingensis, two strict anaerobic beer spoilage bacteria, Int J Food Microbiol. 1997 Mar 18;35(1):29-39.

116. **Tian Y, He X, Torralba M, Yooseph S, Nelson KE, Lux R, (2010):** Using DGGE profiling to develop a novel culture medium suitable for oral microbial communities. Molecular oral microbiology. 25:357-367.

117. **Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007).** The human microbiome project: a strategy to understand the microbial components of the human genetic and metabolic landscape and how they contribute to normal physiology and predisposition to disease. Nature 449: 804–810.

118. **van der Horst J, Buijs MJ, Laine ML, Wismeijer D, Loos BG, Crielaard W, Zaura E. 2013.** Sterile paper points as a bacterial DNA-contamination source in microbiome profiles of clinical samples. J Dent 41:1297–1301.

119. **Vartoukian, S.R., R.M. Palmer, (2007).** "The division "Synergistes"." Anaerobe 13(3-4): 99-106.

120. **Voelkerding KV, Dames SA, Durtschi JD (2009):** Next-generation sequencing: from basic research to diagnostics. Clin Chem. 55:641-658.

121. **Wei Zheng, Maria Tsompana, Angela Ruscitto, Ashu Sharma**. An accurate and efficient experimental approach for characterization of the complex oral microbiota: Microbiome (2015) 3:48DOI 10.1186/s40168-015-0110-9

122. **Wolff, L.F.,** Natural distribution of 5 bacteria associated with periodontal disease. J Clin Periodontol, 1993. 20(10): p. 699-706.

123. **Wrischnik LA, Higuchi RG, Stoneking M, Erlich HA, Arnheim N, Wilson AC (1987):** Length mutations in human mitochondrial DNA: Direct sequencing of enzymatically amplified DNA. Nuclic Acids Res 15:529-542.

124. **Wylie, K. M., Mihindukulasuriya, K. A., Zhou, Y., Sodergren, E., Storch, G. A. & Weinstock, G. M. (2014).** Metagenomic analysis of double-stranded DNA viruses in healthy adults. BMC Biology, 12, 1-10.

125. **Ximenez-Fyvie, L.A., A.D. Haffajee, and S.S.** Socransky, Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol, 2000. 27(9): p. 648-57.

126. **Zambon JJ,** Periodontal diseases: microbial factors. Ann Periodontol. 1996 Nov;1(1):879 -925

127. **Zaura E, Keijser BJ, Huse SM and Crielaard W (2009):** Defining the healthy "core microbiome" of oral microbial Communities. BMC Microbiology 9:259.

128. **Zijnge V, van Leeuwen MB, Degener JE, Abbas F, Thurnheer T, Gmur R (2010).** Oral biofilm architecture on natural teeth. PLoS One 5: e9321.

129. **Zheng , Maria Tsompana, Angela Ruscitto, Ashu Sharma, Robert Genco, Yijun Sun,and Michael J.** Buck, An accurate and efficient experimental approach for characterization of the complex oral microbiota Microbiome (2015) 3:48 DOI 10.1186/s40168-015-0110-9.

Annexures

ANNEXURE I

CONSENT FORM

……..…S/o, w/o, d/o

…….aged about ……..…….years,

Hindu/Christian/Muslim…….residing at

……do solemnly

And state as follows.

I am the deponent herein; as such I am aware of the facts stated here under

I state that I came to Ragas Dental College and Hospital, Chennai for my treatment for

……

…….……I was examined by Dr…….. and I was requested to do the following

1. Full mouth Plaque Score

2. Full mouth bleeding score

3 Measurement of periodontal pocket depth and clinical attachment loss

I was also informed and explained about the collection of plaque during scaling in ……(language) known to me.

I was also informed and explained that the results of the individual test will not be revealed to the public.I give my consent after knowing full consequence of the dissertation/thesis/study and I undertake to cooperate with the doctor for the study.

I also authorise the Doctor to proceed with further treatment or any other suitable alternative method for the study,

I have given voluntary consent to the collection of plaque for approved research.

I am also aware that I am free to withdraw the consent given at any time during the study in writing.

Signature of the patient/Attendant

The patient was explained the procedure by me and has understood the same and with full consent signed in (English/Tamil /Hindi/Telugu?.....) before me

Signature of the Doctor

ANNEXURE II

RAGAS DENTAL COLLEGE & HOSPITAL
(Unit of Ragas Educational Society)
Recognized by the Dental Council of India, New Delhi
Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai
2/102, East Coast Road, Uthandi, Chennai - 600 119. INDIA.
Tele : (044) 24530002, 24530003-06. Principal (Dir) 24530001 Fax : (044) 24530009

TO WHOMSOEVER IT MAY CONCERN

Date: 05/01/2017

From

The Institutional Ethics Board,

Ragas Dental College and Hospital,

Uthandi,

Chennai- 600119

The dissertation topic titled **"Evaluation of the subgingival microbiome in periodontal health and chronic Periodontitis using Next Generation Sequencing Technology"** submitted by **Dr.Kalavani.B.**, has been approved by the Institutional Ethics Board of Ragas dental college and hospital.

RAGAS DENTAL COLLEGE Chennai

Dr. N.S.Azhagarasan,MDS.

Member secretary,

Institutional Ethics Board,

Ragas Dental College and Hospital.

PRINCIPAL
RAGAS DENTAL COLLEGE AND HOSPITAL
UTHANDI, CHENNAI-600 119.